An integrative approach to distinguishing taxonomically challenging microhymenoptera: Eurytomidae (Hymenoptera: Chalcidoidea) associated with cynipid galls on rose

by

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Abstract

Cynipid wasps of the genus *Diplolepis* Geoffrey induce galls exclusively on roses. These galls are susceptible to attack by various hymenopteran parasitoids, the most common of which are members of the family Eurytomidae, with 10 described species having been recorded in association with rose galls in Canada. Because of their small size, sexual dimorphism, morphological plasticity and poorly known biology, species identification of eurytomids is often uncertain. This thesis focused on identifying the eurytomids associated with the galls of the 14 native species of *Diplolepis* in Canada, testing species limits using a combination of morphological, mitochondrial DNA cytochrome *c* oxidase I (*COI*), ecological and geographical data. Six morphospecies were identified using morphological characters, while molecular data identified eight haplogroups.

COI sequences identified one species of *Tenuipetiolus* Bugbee and seven species of *Eurytoma* Illiger, all associated with galls induced by *Diplolepis* in Canada. Inconsistencies were observed when compared to morphospecies, including four haplogroups within the genus *Eurytoma* that were overlooked by morphological studies. The morphospecies *Eurytoma acuta* Bugbee is a junior synonym of *Eurytoma discordans* Bugbee; however, the high intraspecific genetic divergences suggest the existence of a species complex. Two geographically sympatric but genetically distinct haplogroups were found within the morphospecies *Eurytoma spongiosa* Bugbee, "*E. spongiosa* 2" being a new species that was previously overlooked. An identification key along with (re)descriptions of all identified species was provided. This integrative taxonomy approach confirmed the oligophagous nature of most eurytomids, and provides new insight into the life history strategies of these parasitoids.

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Chapter 1: General Introduction

The central unit for biology and its derivatives is the species, as accurate identifications and the establishment of names are pivotal for studies of all biological systems (Padial *et al.* 2010). The concept of species is also among the most controversial topics in biology; however, these disagreements about species concepts are being replaced by a consensus view of species as independent, evolving population-level lineages (de Queiroz 1999). Recently separated lineages are often difficult to determine due to the lack of morphological distinctiveness, reproductive incompatibility, ecological uniqueness or monophyly (de Queiroz 1999), and therefore the use of diverse sources of data and analytical approaches are needed to determine lineage separations known as integrative taxonomy (Dayrat 2005, Padial *et al.* 2010). By avoiding reliance on morphological characters and instead relying more on independent data analytical agreements, integrative taxonomy is the most promising approach to delineating species and testing species hypotheses for problematic groups (Padial *et al.* 2010, Gebiola *et al.* 2012).

Insects are among the most speciose organisms on earth and it has been estimated that there are more than 5 million species (Grimaldi & Engel 2005). Worldwide the largest orders are the Coleoptera (beetles), Lepidoptera (moths and butterflies), Diptera (flies) and Hymenoptera (sawflies, ants, bees and wasps). Distinguishing, naming and cataloguing all these species has always been one of the most challenging tasks for biologists, and the work continues to this day. One order in particular that provides some of the most severe challenges because of their small size (often less than 5 mm in length), is the Hymenoptera (Sharkey 2007, Heraty *et al.* 2011, Gebiola *et al.* 2012). There are an estimated 115,000 species of Hymenoptera, constituting nearly 10 percent of all known organisms (Austin & Dowton 2000, Sharkey 2007).

Hymenoptera have long been recognized as a monophyletic group, and are traditionally divided into two suborders: Symphyta and Apocrita. The paraphyletic symphytans are phytophagous as larvae and the adults do not have a "wasp-waist". Apocrita exhibit a variety of lifestyles, and members of this monophyletic group all have a distinct "wasp-waist" formed by the firm attachment of the first abdominal segment to the thorax, and a sharp constriction between the first and second abdominal segments (Sharkey 2007). Apocrita is further divided into the stinging wasps Aculeata, and Parasitica which includes the majority of the parasitic wasps. A significant portion of hymenopterans are parasitoids, which are insects that undergo larval development by feeding either internally or externally on a single arthropod host, ultimately killing it (Godfray 1994, Quicke 1997). . It has led to an explosive radiation that represents one of the most successful adaptations in the Class Insecta (Grimaldi & Engel 2005, Heraty *et al.* 2011).

As a result of their diverse biology, parasitoids are of ecological and economic importance as regulators of arthropod populations and have been often used in biological control (Whitfield 1998, Austin & Dowton 2000, Heraty *et al.* 2011). However, little is known about the diversity, distribution and biology of most hymenopteran parasitoids due to their small size, complex life cycles and subtle morphological differences between species (Quicke 1997). Mis-identifications of hosts and/or parasitoids are common in the literature, making the host record and distribution of described species unreliable (Santos *et al.* 2011). In addition, many cryptic species complexes exist within Hymenoptera, which are defined as assemblages of closely-related species that have been, or are sometimes classified as one broadly delimited species due to the difficulty of identification on the basis of visible phenotype (Bickford *et al.* 2006, Lumley

& Sperling 2010). These factors present challenges in Hymenoptera when relying solely on morphological characters for species delimitation and identification.

One particularly successful family within Apocrita is the Cynipidae, a family of about 1400 species most of which induce galls on various host plants (Csóka *et al.* 2005). Galls are highly-regulated growth manifestations on plants that serve as a source of high-quality food and shelter from adverse environmental conditions (Shorthouse & Rohfristch 1992, Raman 2011). Galls of most cynipid wasps are susceptible to attack by various species of minute wasps that feed on either the larvae of the inducer or on the tissues of the gall. Insects that feed on the inducers are referred to as parasitoids, and those that feed on gall tissues are referred to as inquilines (Shorthouse 1993). This assemblage of inhabitants associated with a group of galls induced by the same species of cynipid results in complex micro-communities known as component communities, which provide useful models in the study of closed system trophic webs focused on a single resource (Claridge 1987, Stone *et al.* 2002).

The extant members of Cynipidae all belong to the subfamily Cynipinae, which is further divided into 8 tribes, ranging from gall inducers on a variety of plant families to inquilines of other gall inducers (Liljeblad *et al.* 2011). One genus that is responsible for galls commonly observed on the wild roses of northern regions of the world is *Diplolepis* Geoffroy, a small group of cynipids in the tribe Diplolepidini that induce galls exclusively on shrubs of the genus *Rosa* L. (Shorthouse 1993). *Diplolepis* are Holarctic in distribution, with approximately 30 species in the Nearctic Region and 12 species in the Palearctic Region (Plantard *et al.* 1998). Like the component communities associated with other cynipids, such as those of oaks (Stone *et al.* 2002), a variety of microhymenopterans are associated with the galls of *Diplolepis* with parasitoids in

the genus *Eurytoma* Illiger among the most common, often comprising about 40% of total emergents (Shorthouse *et al.* 2005, Shorthouse 2010).

While several studies have been conducted on both adult (Lotfalizadeh *et al.* 2007a, 2007b) and larval (Gómez *et al.* 2011) eurytomids associated with rose gall communities in the Palearctic region, the Nearctic species have received little taxonomic attention. The majority of the Nearctic species were described in a series of publications by Bugbee (1951a, 1951b, 1973); however, many of the original species descriptions are brief, and were often based on a limited number of specimens collected from a single locality. Further, morphological variations from the type specimens were assigned as subspecies, resulting in even more indistinguishable taxa. While identification keys were provided for *Eurytoma* by Bugbee (1951b, 1967), they were based only on females and distinguishing characters used were often ambiguous. The host records for many of the type specimens are absent or limited to a single host, while studies on European species of eurytomids are associated with multiple hosts (Claridge & Askew 1960, Lotfalizadeh *et al*. 2007a). These impediments confound studies on host-parasitoid relationships within galls induced by *Diplolepis* found in Canada (e.g. Shorthouse 2010, Shorthouse *et al.* 2005), and thus a novel approach is needed to delimit these morphologically similar species.

With the advent of molecular taxonomy and phylogenetics, the incorporation of molecular techniques has provided new tools for the study of hymenopteran parasitoids. One of the molecular tools used for species identification is the DNA barcoding, which uses a short fragment of 658 base pairs at the 5' end of the cytochrome *c* oxidase subunit I (COI) gene as a universal "barcode" for species identification (Hebert *et al.* 2003). Eurytomids are taxonomically challenging due to their small size and structural homogeneity, thus an ideal

candidate for DNA-based identification. Elsewhere, Li *et al.* (2010) successfully used COI to distinguish morphologically similar eurytomids of the genus *Sycophila* Walker that are associated with figs in China into genetically distinct haplogroups, and match sexually dimorphic conspecific males and females. Lotfalizadeh *et al.* (2007a) used a combination of morphological and COI data to delimit a new species, *Eurytoma caninae* Lotfalizadeh & Delvare, a previously overlooked species of *Eurytoma* associated with rose galls in West Palearctic.

The primary goal of this thesis is to use an integrative taxonomy approach to identify parasitoids of the family Eurytomidae associated with galls induced by cynipid wasps of the genus *Diplolepis* found on various species of roses across Canada. In Chapter two, morphological, molecular, ecological, and geographical data were used to delimit eurytomids associated with galls of 14 species of *Diplolepis* in Canada by testing congruency of genetic variation, morphological differences, host specificity, and geographical distribution between different populations. It was predicted that a detailed study of the eurytomids associated with galls induced *by Diplolepis* in Canada, using an integrative taxonomy approach, would reveal that past designations of species are weak and that revisions are necessary. It was hypothesized that if the eurytomids associated with galls of *Diplolepis* are oligophagous generalists rather than monophagous specialists, then the series of eurytomids associated with galls of *Diplolepis* would be comprised of synonyms and new species and that the host ranges of Canadian eurytomids would be far greater than currently known. In Chapter three, formal redescriptions and updated range maps of the eurytomid species associated with rose gall wasps in Canada are provided along with a dichotomous key for both males and females.

Biology of *Diplolepis*

By manipulating the growth and physiology of their host plants, gall inducers stimulate the production of highly nutritious plant cells normally not found in the attacked organ (Bronner 1992). With roughly 1300 described species, the family Cynipidae is the second largest group of gall inducers after the gall midges Cecidomyiidae (Ronquist & Liljeblad 2001, Csóka *et al.* 2005). Capable of inducing structurally complex and morphologically distinct galls, described cynipids can frequently be identified in the field using gall morphologies (Csóka *et al.* 2005). These nutritive cells are the sole source of food for the inducer larvae, which are continually produced as the older cells are consumed (Shorthouse 2010). In addition to acting as a source of food, gall tissues also provide a shelter that offers protection from adverse weather and predation (Stone and Schönrogge 2003).

Cynipids of the genus *Diplolepis* are univoltine, producing only one generation per year. While the majority of their life cycle is spent as immatures inside the chamber of their galls in which they overwinter, the short flight periods of the adults are synchronized with the availability of host tissues the following year at a specific stage for oviposition and gall initiation (Shorthouse 2010). The precise deposition of eggs within specific plant organs has been hypothesized as the key factor contributing to specific-specific differences in gall structure, and has thus influenced the radiation of the genus (Shorthouse *et al.* 2005).

Wild roses are notoriously difficult to identify due their morphological variability and hybridization, including the 12 endemic species found in Canada (Shorthouse 2010). Galls of *Diplolepis* are commonly collected in Canada from *Rosa acicularis* Lindl., *Rosa arkansana* Porter, *Rosa blanda* Gray, and *Rosa woodsii* Lindl. (Shorthouse 2010), and all 14 species of Canadian *Diplolepis* induce morphologically distinct galls regardless of the host plant (Table

1.1). *Diplolepis* are host-specific and often only attack one or two endemic species of rose, although host shifts to introduced rose species have been reported (Shorthouse 1988, Shorthouse & Brooks 1998).

Biology of Communities associated with galls induced by *Diplolepis*

Despite being apparently well-defended by gall tissues, gall inducers usually support richer communities and suffer higher mortality than externally feeding herbivores (Hawkins 1988, Price & Pschorn-Walcher 1988). With the combined effects of inquilines and parasitoids, the mortality rate of gall inducers in some populations can be up to 90% (Shorthouse 1993, Stone *et al.* 1995, Shorthouse *et al.* 2005). Approximately 200 species of hymenopteran parasitoids are known to be associated with cynipid galls in the Northern Hemisphere (Csóka *et al.* 2005). Many of the parasitoids associated from cynipid galls are host-specific, and individual parasitoid species generally only attack galls induced by members of a single tribe of cynipid gall wasps (Csóka *et al.* 2005). Many of the parasitoids in cynipid galls are solitary koinobiont ectoparasitoids whose larvae feed suctorially and externally on their host, although various species of Eurytomidae and Torymidae have been observed to also feed on gall tissue (Shorthouse 1998, Csóka *et al.* 2005, Gómez *et al.* 2011).

Inquilines of the genus *Periclistus* Förster are also commonly found within galls of *Diplolepis*, which kill the inducer when they deposit eggs on the inner surface of the chambers (Shorthouse 1997). Both *Diplolepis* and *Periclistus* larvae are targeted by many specialized hymenopteran parasitoids, and extensive work has been done on the insect communities (herein referred to as rose gall communities) associated with *Diplolepis* galls in Canada (e.g. Shorthouse 1973, 1993, Judd 1989, Brooks and Shorthouse 1997). Aside from *Orthopelma* Taschenburg

(Ichneumonidae), which is a member of the superfamily Ichneumonoidea, all other parasitoids associated with galls of *Diplolepis* belongs to the large superfamily Chalcidoidea. In total, 10 genera of chalcids belonging to six families have been recorded, including Eulophidae, Eupelmidae, Eurytomidae, Ormyridae, Pteromalidae and Torymidae (Shorthouse 2010).

Eurytomids Associated with Galls Induced by *Diplolepis*

Members of the family Eurytomidae (Hymenoptera, Chalcidoidea) are predominantly endophytic, either as phytophages or as parasitoids of phytophagous insects (DiGiulio 1997, Gómez *et al.* 2011). They are generally recognized by their black, non-metallic body, heavily punctured sculpturing and the rectangular, collar-like pronotum (Noyes 2012). Females (Fig. 1.1) are generally larger with a shorter petiole and larger metasoma than their male conspecifics (Fig. 1.2). The antennae of eurytomids are also sexually dimorphic; antennae of females are moniliform (Fig. 1.1) whereas those of males are pedicellate (Fig. 1.2).

Eurytomids exhibits a wide range of biologies, ranging from parasitoids to seed eaters and gall inducers (Lotfalizadeh *et al.* 2007b). Due to their diverse feeding habits, they are of economic importance, including both pestiferous species of cultivated plants and those used as biological control agents (Plaut & Mansour 1973, Zerova & Fursov 1991, Neser 2008, Mena-Correa *et al.* 2010). Like many other parasitic wasps, the identification of eurytomids is difficult due to their small size, high level of intraspecific morphological diversity and convergence of morphological traits (Lotfalizadeh *et al.* 2007b, Gebiola *et al.* 2012). The monophyly of Eurytomidae has always been controversial, as no synapomorphies defining the family are found

using either molecular (Chen *et al.* 2004, Munro *et al.* 2011) or morphological (Lotfalizadeh *et al.* 2007b, Gates 2008) analyses. The subfamily Eurytominae is the largest and the least studied of the three subfamilies of Eurytomidae (Stage & Snelling 1986), including the polyphyletic genus *Eurytoma* Illiger in which over half of the species of eurytomids belong (Chen *et al.* 2004, Lotfalizadeh *et al.* 2007b). In the most recent revision of Eurytominae by Lotfalizadeh *et al.* (2007b), species with a carinate gena (Fig 1.3) and that show no other outstanding characters were redefined as *Eurytoma sensu stricto* and divided into 11 species groups (Fig 1.3). All eurytomids of gall-inducing cynipids are grouped within the *rosae* species group (Lotfalizadeh *et al.* 2007b), supported by the presence of postgenal depression (Fig 1.3) and the precoxal tooth formed by the raised adscrobal carina (Fig. 1.4).

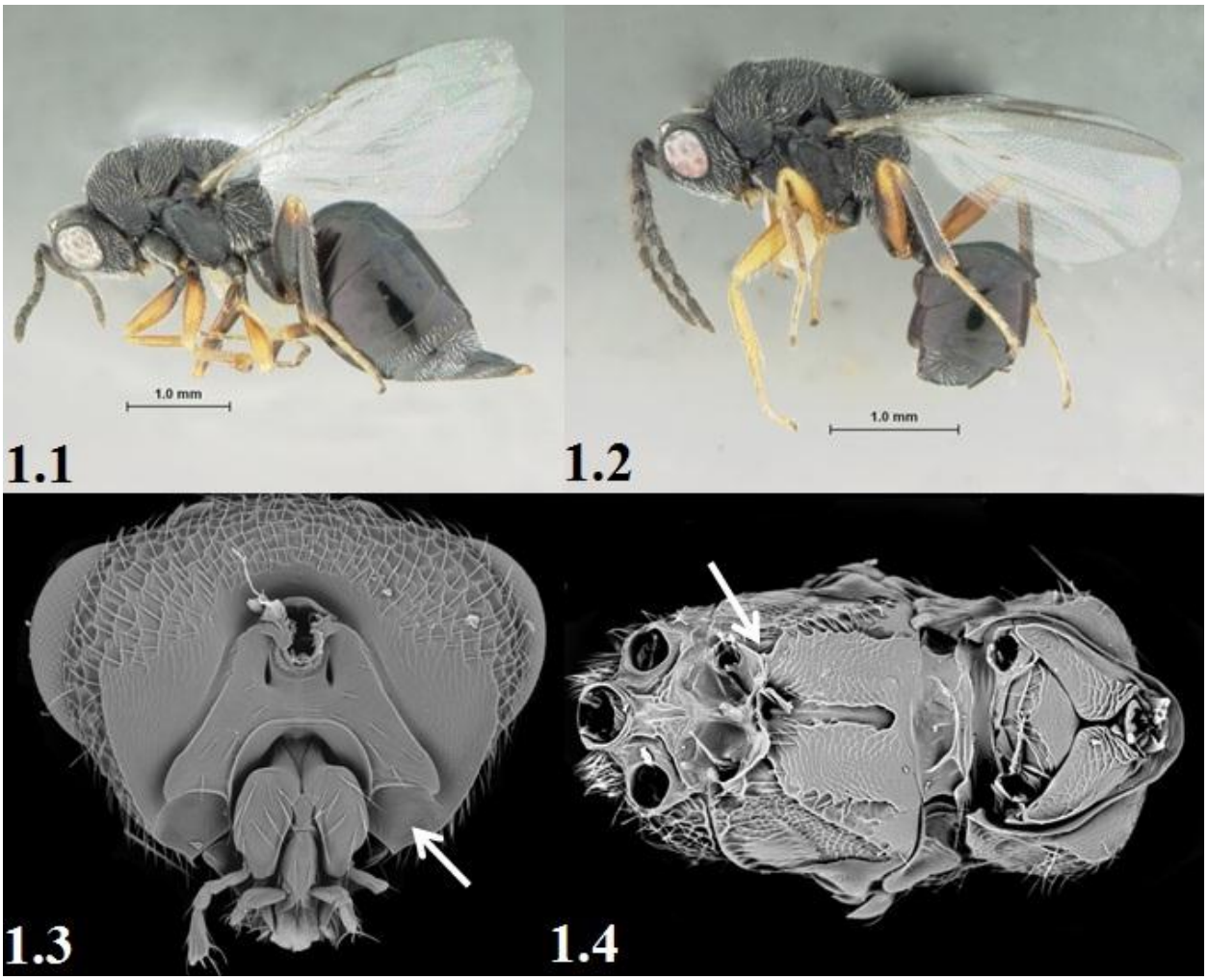
Eurytomids are frequently the most common parasitoid in gall communities (Shorthouse *et al.* 2005, Noyes 2012). While the majority of eurytomids are largely univoltine, bivoltine species have been recorded (Shorthouse 2010). The flight time of adult eurytomids corresponds to the stage of development of the galls in which they must oviposit (Shorthouse 2010). Adult females have a narrow window of opportunity in which to oviposit into developing galls before the inducer larvae becomes enveloped by gall tissues (Fig. 1.5, Eggs in Fig. 1.6) and beyond the reach of the *Eurytoma* ovipositor. The developing larvae are koinobiont ectoparasitoids of either inducers (Fig. 1.7) or inquilines, including entomophytophagous species whereby a parasitoid initially consumes its insect host and then completes its development by consuming surrounding plant tissues (Zerova & Fursov 1991, Leggo & Shorthouse 2006). When the larvae mature, they pupate and overwinter within the gall before exiting the following year in search of new hosts (Fig. 1.8).

DNA Barcoding

DNA barcoding is a molecular and bioinformatics tool that utilizes the variability within a single standard molecular marker to discriminate biological entities (Casiraghi *et al.* 2010). One of the major benefits of a DNA barcode is the possibility of easily associating all life stages and sex, as well as identifying organisms from parts/pieces of the organism (e.g. Li *et al.* 2010, Rougerie *et al.* 2011). DNA barcodes have been used to link unknown specimens with reference sequences of previously identified voucher specimens on the Barcode of Life Data System (BOLD, www.boldsystems.org, Ratnasingham & Hebert 2007) and thus provide rapid and accurate identifications (Hebert *et al.* 2003). While the exclusive use of the single DNA sequence region COI in species identification has been criticized by some (e.g. Meier *et al.* 2006, Taylor & Harris 2012), the use of DNA barcodes in combination with other genetically determined characters (ecological, behavioural, or morphological) has become an effective technique for identification at the species level as well as revealing cryptic diversity in a number of studies covering a range of taxa (e.g. Hebert *et al.* 2004, Smith *et al.* 2006, 2007, Rivera & Currie 2009, Babin-Fenske *et al*. 2012). In addition, COI has also been used extensively to test host specificity and geographical variation among hymenopteran groups (e.g. Sheffield *et al.* 2009, Ács *et al*. 2010, Nicholls *et al.* 2010, Gebioloa *et al.* 2012), including eurytomids (Lotfalizadeh *et al.* 2007a, Li *et al.* 2010).

Table 1.1. Species of *Diplolepis* and their galls in Canada (modified from Shorthouse 2010).

Species	**Host**	**Organ Attacked**			**Chambers**		**Season Gall Initiated**		
		Leaves	Stems	Adventitious Shoots	Single	Multi	Spring	Mid-Summer	Late-Summer
D. bassetti (Beutenmuller)	*R. woodsii* Lindl.	X			X		X		
D. bicolor (Harris)	*R. arkansana* Porter *R. blanda* Gray *R. woodsii*	X			X		X		
D. fusiformans (Ashmead)	*R. blanda* *R. woodsii*		X			X		X	
D. gracilis (Ashmead)	*R. acicularis* Lindl.	X			X			X	X
D. ignota (Osten Sacken)	*R. arkansana*	X			X				X
D. nebulosa (Bassett)	*R. blanda* *R. woodsii*	X			X		X	X	X
D. nodulosa (Beutenmuller)	*R. blanda* *R. arkansana* *R. woodsii*		X		X		X		
D. polita (Ashmead)	*R. acicularis* *R. nutkana* Presl. *R. woodsii*	X			X		X		
D. radicum (Osten Sacken)	*R. acicularis* *R. blanda* *R. woodsii*			X		X	X		
D. rosaefolii (Cockerell)	*R. acicularis* *R. arkansana* *R. blanda* *R. nitida* Willd. *R. virginiana* Mill. *R. woodsii*	X			X			X	X
D. spinosa (Ashmead)	*R. blanda* *R. rugosa* Thunb. *R. woodsii*		X			X	X		
D. triforma Shorthouse & Ritchie	*R. acicularis* *R. canina* L.		X			X	X		
D. tumida (Bassett)	*R. woodsii*		X			X	X		
D. variabilis (Bassett)	*R. woodsii*	X			X	X		X	X



Figures 1.1–1.4. Typical *Eurytoma* within the *rosae* species group: 1.1, Adult female habitus; 1.2, Adult male habitus; 1.3, Posterior head capsule showing carinate gena, postgenal depression indicated by arrow; 1.4, Mesosoma in ventral view, precoxal tooth indicated by arrow.

Figures 1.5–1.8. Life stages of a eurytomid associated with rose galls: 1.5, Adult female ovipositing into a developing gall induced by *Diplolepis polita*; 1.6, Egg attached to the inside wall of a larval chamber. 1.7, Mature eurytomid lava with frass. 1.8, Pupal stage in a gall ready to overwinter. (Photo credits: Brandy L. Fenwick).

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Chapter 2 - Testing species limits of eurytomids (Hymenoptera: Eurytomidae) associated with galls induced by *Diplolepis* (Hymenoptera: Cynipidae) in Canada using integrative taxonomy.

Abstract

The study of insect host-parasitoid relationships are often confounded by the difficulties associated with species delimitation in taxonomically difficult groups. Integrative taxonomy uses multiple lines of evidence such as morphological, molecular, ecological and geographical data to test species limits. Eurytomids (Hymenoptera: Eurytomidae) are the most common parasitoids associated with rose galls of *Diplolepis* (Hymenoptera: Cynipidae) in Canada. They are difficult to identify due to their small size, morphological conservatism, and unreliable published host records. This study tests the species limits of eurytomids associated with galls induced by *Diplolepis* in Canada using an integrative taxonomy approach utilizing adult morphological, mitochondrial gene COI, host record and geographical range. Incongruences between morphological and molecular data were found within the *Eurytoma discordans* complex, as three morphospecies were shown to be synonymous. The results also revealed the presence of cryptic species within *Eurytoma spongiosa*. Furthermore, issues that have long impeded the ecological and biological studies on eurytomids on rose galls such as host specificity and sex association were readily resolved using the integrative taxonomy approach, providing new insights into the evolutionary history of this taxonomically difficult group.

Introduction

Gall wasp communities are important model systems in studies on trophic interactions among insects, and species level identification of community members is required for analysis of food webs (Gómez *et al.* 2011). One of the driving forces behind gall parasitoid speciation is the phenological patterns of their hosts, as they can only reach their hosts by oviposition through gall tissues, which proliferates as they develop (reviewed in Csóka *et al.* 2005). Only a short ovipositor is required to reach the host early in gall development, but the host egg or young larva only provides resource either for small idiobiont parasitoids or koinobionts (Csóka *et al.* 2005), thus longer ovipositors are required to reach host larvae later in gall development. Several studies have also shown that gall location on the host plant and the time required for gall development both affect parasitoid species richness (e.g. Plantard *et al.* 1996, Plantard *et al.* 1999, Schönrogge *et al.* 1995, 1996); however, it is unclear why a specific parasitoid attacks one host and not another that is present on the same host plant in the same place at the same time (Csóka *et al.* 2005). All known parasitoids that attack cynipid hosts are wasps, including members from the superfamily Ichneumonoidea and Chalcidoidea (Csóka *et al.* 2005).

The superfamily Chalcidoidea contains an estimated 500,000 species, making it one of the largest biologically and morphologically diverse groups of parasitic wasps (Gibson *et al.* 1997, 1999, Munro *et al.* 2011). While some chalcidoids are phytophagous, the majority are entomophagous (Gibson *et al.* 1997). Their hosts include all life-history stages of 12 orders of insects, two orders of arachnids and even one family of nematodes (Gibson *et al.* 1999). Chalcids within the family Eurytomidae has over 1400 nominal species in 84 genera and are found in all zoogeographical regions (Gates 2008, Lotfalizadeh *et al.* 2007b, Noyes 2012).

Eurytomids are largely endophytic as seed feeders, gall inducers or parasitoids of phytophagous insects (Lotfalizadeh *et al.* 2007b). The accurate identification of eurytomids, in particular the large genus *Eurytoma* Illiger, which includes more than 700 described species and consists over half of all known eurytomids, has been proven difficult using existing morphological keys due to overlapping diagnostic characters and lack of illustrations (e.g. Bugbee 1967). As a result, phylogenetic, ecological or evolutionary studies of eurytomids have been impeded. The degree of morphological conservatism is particularly prominent in members of the *Eurytoma rosae* species group, which parasitizes various gall inducing cynipids, tephritids and curculionids (Lotfalizadeh *et al.* 2007b). Members of the *rosae* group are often morphologically similar and impossible to segregate into morphospecies, despite being ecologically and genetically distinct (Ács *et al.* 2002, Lotfalizadeh *et al.* 2007a, Gómez *et al.* 2011).

Among the parasitoids associated with cynipid wasps of the genus *Diplolepis* Geoffroy, eurytomids are the most common, comprising about 40% of total emergents (Shorthouse *et al.* 2005, Shorthouse 2010). A total of 14 native species of *Diplolepis* (Hymenoptera, Cynipidae) species have been recorded in Canada, all of which induce structurally distinct galls on *Rosa* (Shorthouse 2010). These rose galls are heavily attacked by chalcid parasitoids, of which eurytomids are the most abundant (Shorthouse *et al.* 2005, Shorthouse 2010). Ten species of eurytomids are known to be associated with galls of *Diplolepis* in Canada (Noyes 2012), feeding as koinobiont ectoparasitoids of either the inducer or cynipid inquilines of the genus *Periclistus* Förster (Noyes 2012). *Periclistus* are cynipids that have lost the ability to initiate galls but have retained the ability to modify the anatomy of their host galls (Shorthouse 1998). While most eurytomids are univoltine and overwinter within the gall before exiting the following spring, cases of fall emergence have been recorded where mature eurytomids pupate and exit the gall in

the fall of the year of gall initiation (Brooks & Shorthouse 1997, Shorthouse 2010). Subtle morphological differences, sexual dimorphism and poor host records have provided challenges in accurate identification of eurytomids using existing identification keys, confound studies on host-parasitoid relationships within galls induced by *Diplolepis* found in Canada (e.g. Shorthouse 2010, Shorthouse *et al.* 2005), and thus a novel approach is needed to delimit these morphologically similar species.

With the advances in molecular biology, the use of molecular markers has proven essential for delimiting closely-related species among hymenopteran parasitoids (Heraty 2009, Santos *et al.* 2011). The mitochondrial genome in particular serve as a good model for the study of molecular evolution and population genetics, with high rates of evolution and genome reorganization observed in known chalcid wasp genomes (Dowton & Austin, 1995, Oliveira *et al.* 2008, Xiao *et al.* 2011). A short fragment of the mitochondrial cytochrome *c* oxidase I (COI) is the core DNA barcoding animal gene and has demonstrated success in species identification (e.g. Hebert *et al.* 2003, Rivera & Currie 2009, Robinson *et al.* 2009, Jeffery *et al.* 2011). In addition, distinct molecular clades or haplogroups have been used in the identification of morphologically cryptic taxa (e.g. Hebert *et al.* 2004, Smith *et al.* 2006, 2007, Babin-Fenske *et al.* 2012). However, controversy exists on the exclusive reliance of mtDNA in species delimination without the inclusion of morphological or ecological datasets (e.g. Cognato 2006, Meier *et al.* 2006). Thus, an integrative taxonomy approach is needed using multiple independent character data sources and avoiding reliance on key characters alone in testing species hypotheses for problematic groups (Dayrat 2005, Will *et al.* 2005, Padial *et al.* 2010, Gebiola *et al.* 2012). COI has been shown to be a valuable tool in identifying cryptic taxa, in combination with morphological and ecological data, for testing host-specificity and geographical variability for

Hymenoptera (e.g. Smith *et al.* 2008, Sheffield *et al.* 2009, Ács *et al.* 2010, Kaartinen *et al.* 2010, Sun *et al.* 2011, Gebioloa *et al.* 2012) including members of Eurytomidae (Lotfalizadeh *et al.* 2007a, Li *et al.* 2010).

The purpose this study was to use an integrative taxonomy approach to delimit eurytomids associated with galls of 14 species of *Diplolepis* in Canada by testing congruency of genetic variation, morphological differences, host specificity, and geographical distribution between different populations. Additionally the validity of species limits proposed by Bugbee was tested using COI sequences.

Materials & Methods

Sample collection

Maturing or mature galls were collected from various sites in Ontario and Québec from 2009–2011, either in the spring after snow melt for galls induced the previous year, or in the fall after galls had matured. Galls from the previous year were stored in jars at room temperature allowing the inhabitants, such as eurytomids, to exit the galls. Galls collected in the fall were subjected to -5°C for 3–4 months to break diapause. All inhabitants, including the eurytomids were aspirated or removed from the jars with a paint brush, and then stored in 100% ethanol. Each collection was given a unique collection number such that the inhabitants were associated with a collection of galls induced by the same species of *Diplolpeis* from the same location. Additional specimens were obtained from the Joseph Shorthouse Collection, a research collection of galls induced by *Diplolepis* and associated inhabitants at Laurentian University, in

Sudbury, Ontario including inhabitants from all 14 native *Diplolepis* species collected across Canada. The specimens used for this study were limited to those with sufficient ecological and geographical data to unambiguously identify host galls (n = 423). Eurytomids were selected from pinned specimens and bulk samples stored in 100% EtOH and identified to the species level based on dichotomous morphological keys by Bugbee (1951a, 1967) in combination with host records whenever possible. Specimens that could not be confidently identified were separated into distinct morphopecies. Localities of the eurytomids used in the study are shown in Fig. 2.1. This map was generated using Simplemappr (Shorthouse 2012, www.Simplemappr.net). Detailed specimen records can be found in Appendix I.

DNA extraction and PCR amplification of COI barcoding region

DNA voucher specimens were chosen from specimens collected from 1998–2011. DNA extractions were performed at the Canadian Centre for DNA Barcoding (CCDB) in Guelph, Ontario using a silica-based 96-well automated extraction according to the protocol described by Ivanova *et al.* (2006, 2007) in combination with the non-destructive voucher retrieval method described in Porco *et al.* (2010) using a series of primers listed in Table 2.1. PCR amplification and sequencing were performed according to the standard protocol used by CCDB (Ivanova & Grainger 2007a, 2007b).

Phylogenetic Inference

Contigs were assembled using Sequencher version 4.5 and aligned by CLUSTALX in MEGA version 5.05 (Tamura *et al.* 2011) and by eye. Genetic distances were calculated using the Barcode of Life Data System (BOLD) using Kimura-2-Parameter (Ratnasingham & Hebert

2007, http://www.boldsystems.org). Sequences of eurytomids with >350bp were used in all analyses, with the sequences of *Orthopelma mediator* Thunberg (Ichneumonidae), *Ormyrus rosae* Ashmead (Ormyridae) and *Torymus bedeguaris* (L.) (Torymidae) as outgroups.

Maximum likelihood (ML) analyses were performed using the Kimura 2-Parameter (K2P) distance model (Kimura 1980) in MEGA 5.05 and visualized as a phylogenetic tree. Branch support was assessed with 1,000 bootstrap pseudoreplicates and was considered as supported when bootstrap value was >70%. Similarly, Bayesian inference (BI) using gamma-distributed rate variation across sites and a proportion of invariable sites with HKY+I+G model, as selected by JModeltest version 0.1.1 (Posada 2008) was performed using MrBayes 3.2 (Ronquist *et al.* 2012). Two parallel runs of four simultaneous Monte Carlo Markov chains (three heated and one cold) were run for four million generations, and trees sampled every 1,000 generations. The burn-in value was set at 25% of the total sampled topologies, with the phylogeny estimated from a majority-rule consensus of the remaining trees at the threshold for clade acceptance set at 0.95. The trace files sequences and specimen information are deposited in the project Eurytomidae associated with galls of *Diplolepis* in Canada (project code MZEDO) on BOLD (http://www.boldsystems.org/).

Morphological Study

Morphospecies were compared to the voucher specimens used for molecular study *a posteriori*, and sorted according to haplogroups. These vouchers were chemically dried using HMDS (Heraty & Hawks 1998) before being point or card mounted. Scanning electronic microscopy (SEM) or stereomicroscope photographs were taken using methods described by Gates and Pérez-Lachaud (2012). The vouchers were also compared with type specimens

located in the National Museum of Natural History (USNM) in Washington D.C., USA or the Canadian National Collection of Insects (CNCI) in Ottawa, Ontario.

Results

COI Species Delimitation

COI sequences were obtained from 220 of 423 specimens; the specimens that failed to generate sequences were excluded from phylogenetic analyses. Sequence lengths ranged from 223bp – 632bp, with the majority around 400bp and show a strong A+T nucleotide bias (mean = 0.752) in comparison to C+G (mean = 0.248). Phylogenetic analyses identified eight haplogroups of eurytomids, seven of which have successfully matched identified females with male conspecifics that were morphologically unidentifiable. All haplogroups were well supported by maximum likelihood bootstrap and Bayesian posterior probabilities (Fig 2.2). An expanded phylogenetic tree can found in Appendix II. Both the subfamily Eurytominae (*Tenuipetiolus* + *Eurytoma*) and the genus *Eurytoma* were recovered as monophyletic, and *Eurytoma iniquus* Bugbee, *Eurytoma longavena* Bugbee and *T. ruber* were recovered as distinct clades (Table 2.3). Deeply divergent lineages were revealed in the recognized morphological species *Eurytoma spongiosa* Bugbee, including an additional clade "*E. spongiosa* 2" that is morphologically similar from the nominal *E. spongiosa* but exhibits differences in host preference (Fig. 2.2, Table 2.3). *Eurytoma discordans* Bugbee, *Eurytoma acuta* Bugbee and *Eurytoma calcarea* Bugbee were grouped together into one genetically variable clade, with overlapping geographical range and host records (Table 2.3). In addition, two rare haplogroups were revealed among unidentified species, *Eurytoma* sp. 1 with 4 specimens in association with galls of *D. nodulosa* and *D. rosaefolii* found in Ontario (Fig. 2.2, Table 2.3), and a single male

specimen in association with galls of *D. ignota* in Alberta as *Eurytoma* sp. 2 (Fig. 2.2, Table 2.3). The intra-specific variation ranged from 0.2% to 3.8%, whereas the inter-specific divergence was 5.7%–20.2% (Table 2.4).

Morphological Study

In total, 8 morphospecies of eurytomids were found associated with galls induced by *Diplolepis* from across Canada, including five of the ten species previously known from Canada (Table 2.2). *Eurytoma obtusilobae* Ashmead was only observed in one collection from galls of *D. radicum* found near Kelowna, BC in 1999. These specimens failed to generate sequences and were not used for this study. With the exception of *Tenuipetiolus ruber* Bugbee, all other seven morphospecies belong to the genus *Eurytoma* within the *rosae* species group, characterized by the presence of postgenal depressions and the raised adscrobal carina which forms the precoxal tooth in front of the mid-coxal cavities in lateral view (Lotfalizadeh *et al.* 2007a).

Key morphological characteristics traditionally used to distinguish eurytomids were found to be ambiguous due to subtle variation from the type specimens. Significant morphological variation was often correlated with size, including the absence or atrophy of key characters in the smaller specimens. Other variation included colour of the scape, fore- and midlegs (yellow to black) and the ratio of wing vein length. New characters such as the number and arrangement of multiporous plate sensilla, and sculpturing on the petiole were useful in distinguishing male specimens, many of which were previously unidentifiable if encountered singly.

Host and Geographical Records

Ten new provincial records were established as a result of this study (Table 2.3), including a new Canadian record for *E. iniquus*. In addition 18 new host associations have been discovered, greatly expanding the known host record in North America. With the exception of *Eurytoma* sp. 2, which has only been observed in association with galls of *D. ignota*, all other haplogroups are associated with 2–6 different hosts (Table 2.5). In addition 2 generations of *E. longavena* and *E. spongiosa* 2 were collected, from both spring (e.g. *Diplolepis polita*) and fall initiated galls (e.g. *Diplolepis nebulosa*).

Discussion

Testing species limits using COI

Accelerated rates of evolution of the chalcid mitochondrial genome have been correlated with parasitic lifestyles; however, testing the species limits of recently diverged lineages is difficult because the organisms often had insufficient time for the evolution of diagnostic characters or complete reproductive isolation (Xiao *et al.* 2011, Gebiola *et al.* 2012). Independent lines of evidence were used in the testing of species limits, thus avoiding the reliance of one particular dataset.

The COI sequences resolved closely related species of eurytomids that are difficult or impossible to distinguish morphologically. This first "screening" of morphospecies using COI eliminated misidentification due to phenotypic plasticity and drew attention to problematic clades that required further investigation (Li *et al.* 2010). An additional benefit of molecular

analyses is the ability to associate sexually dimorphic eurytomids in a simple and precise way, where males were previously unknown or indistinguishable due to poor species descriptions. The results of this study clearly show the COI sequences are taxonomically informative and species boundaries defined by deep COI divergences are incongruent with morphological studies by Bugbee (1951a, 1951b, 1967, 1973).

The species limits of three of the eight haplogroups were clearly resolved by COI sequences although the other five haplogroups showed conflicting results with existing morphological data (Bugbee 1951b, 1967). Deep phylogenetic splits within the COI data support the existence of cryptic genetic species in *Eurytoma*, consistent with previous studies of eurytomids of Palearctic gall communities (Ács *et al.* 2002, Lotfalizadeh *et al.* 2007a, Gómez *et al.* 2011). As the two *E. spongiosa* species largely attack the same galls synchronically and sympatrically, a possible hypothesis for the lack of consistent morphological differences between the two species is the presence of *Wolbachia*, a common and widespread group of intracelluar bacteria found in the reproductive organs of arthropods that can cause cytoplasmic incompatibility (reviewed in Werren *et al.*, 2008). *Wolbachia* bacteria have evolved various mechanisms for manipulating the reproduction of their hosts, including progeny sterility or mortality when mating occurs between infected males and uninfected females or females infected by a different strain (Werren *et al.* 2008). *Wolbachia* bacteria have also been considered for confounding DNA based identification as individuals within a population being swept with a different *Wolbachia* strain may appear as different species (Smith *et al.* 2012). While studies have shown that the presence of *Wolbachia* DNA is unlikely to compromise the accuracy of the DNA barcode library (Smith *et al.* 2012), their presence is much higher within Hymenoptera in comparison to other groups of arthropods tested and has been hypothesized to be the cause of

host speciation in parasitic Hymenoptera (Bordenstein *et al.* 2001, Sun *et al.* 2011). While the co-existence of cryptic generalist parasitoids has been reported in cynipid galls (Lotfalizadeh *et al.* 2007a, Nicholls *et al.* 2010), further screening for *Wolbachia* and investigation of the cause of reproductive isolation may help to delimit the two *E. spongiosa* species.

Eurytoma acuta, *E. calcarea* and with *E. discordans* were described as morphologically distinct from each other based the colour of the scape and shape of the stigma club and marginal vein (Bugbee 1951b). Based on specimens examined in this study, these characters were shown to be extremes of an evolutionary continuum rather than stable characters and are thus unreliable as diagnostic characters. The three species were also previously distinguished by their range and host, which has been expanded and now overlap as a result of this study. The high intraspecific divergence rate of this clade suggests the presence of a species complex, where retention of ancestral polymorphism and hybridization may have resulted in the failure of molecular tracing of species boundaries (Li *et al.* 2010). Hence, *E. acuta* and *E. calcarea* should be synonymized, under the more senior name *E. discordans* pending further revision of this species complex.

Host Specificity

The new distribution and host records and host suggest that eurytomids exhibit a much wider host range than previously reported (Noyes 2012), as the majority of eurytomid species are either oligophagous or polyphagous and are observed wherever their hosts occur. Thus, using host records and range as key characteristics (Bugbee 1951b, 1967, 1973) in species delimitation is likely error-prone. The presence of fall emergents in *E. longavena* and *E. spongiosa* 2 in both

spring and late summer initiated galls strongly suggests that these species are bivoltine, where the first generation emerges in the spring and attacks freshly initiated galls, while the second generation develops and exits from these spring galls in the fall to attack the galls of *Diplolepis* that are maturing. Bivoltine parasitoids are also generally smaller in size, as both *E. longavena* and *E. spongiosa* are smaller in comparison to larger species (e.g. *E. discordans*), which have only a single generation each year.

The radiation of the *Diplolepis* species onto novel host plants and organs is likely in response to selection for exclusion of natural enemies (enemy-free space) such as the eurytomids (Stille 1985, Price *et al.* 1995, Stone *et al.* 2002). Most eurytomids are found across a wide geographical range and on multiple rose species, their natural range likely expands parallel to their hosts. For instance, in cases of *Diplolepis* shifting hosts (Shorthouse 1988, Shorthouse unpublished data), the same species of eurytomids are found attacking galls on the new host plant. In a study by Nicholls *et al.* (2010), strong evidence was provided for oak gall parasitoids that have tracked their hosts through space and time, showing radiation into cryptic species together with host radiations at multiple trophic levels. It is likely that eurytomids associated with rose galls also have stable, long-term co-evolutionary interactions with other species in this community, responding as a single unit to environmental perturbations (Nicholls *et al.* 2010).

Species of eurytomids examined for this study showed a close evolutionary relationship with their hosts, often attacking hosts inducing galls on a specific plant organ. *Eurytoma longavena* was observed almost exclusively in single-chambered galls such as those induced on leaves (e.g. *D. polita*). The only exception was the galls induced by *D. fusiformans* (Ashmead), a small, single-chambered stem gall that is closely related to the other basal lineages of leaf-gall

inducing species (Plantard *et al*. 1998). Likewise *E. discordans* was found in multi-chambered stem galls where it sometimes consumes several hosts by tunneling from one larval chamber to another (Brooks & Shorthouse 1997). *E. discordans* was recorded in single-chambered galls induced by *D. bicolor*, although these conspecifics are smaller likely due to their smaller host. *Tenuipetiolus ruber* was rarely found in galls of *Diplolepis polita* and *Diplolepis triforma* In addition to *Diplolepis*, this species has also been found in association with cynipid galls on blackberry induced by *Diastrophus* (Bugbee 1951a) and is a generalist parasitoid with a host range that likely includes other cynipids.

The two unidentified species of *Eurytoma* and *E. iniquus* were collected from galls with high levels of inquilinism by *Periclistus* (Table 2.5). *Eurytoma nigricoxa* is the only species in Canada that has been recorded in association with *Periclistus* modified galls (Bugbee 1967); however, none of the three species matches *E. nigricoxa* upon comparison with the holotypes. Additionally, as the holotype of *E. nigricoxa* lacks host information, the likelihood of *E. nigricoxa* being associated with rose galls is low given the diversity and wide host range of eurytomids. It is likely these three *Eurytoma* species are parasitoids of *Periclistus*; although more specimens are needed to further investigate these host relationships.

This study has established a DNA barcode reference library for eurytomids, particularly *Eurytoma* associated with galls of *Diplolepis* in Canada. In fact, this is the first phylogenetic study of the Nearctic *Eurytoma* and results suggest that many eurytomid species associated with rose galls (Bugbee 1951a, 1951b, 1967, 1973) deserve further investigation. It is also clear that COI sequences are needed to determine the validity of the rare species (e.g. *E. obtusilobae*). Detailed studies of the two *E. spongiosa* species and the *E. discordans* species complex will

undoubtedly aid in the delineation of species. Eurytomid larvae have been shown to be morphologically informative (Claridge & Askew 1960, Henneicke *et al.* 1992, Gómez *et al.* 2011), therefore linking larvae with adults using COI will also provide valuable information on host preference. The potential of using COI in addition to other datasets to identify all life stages and both sexes greatly facilitates the identification capabilities of eurytomids.

The presence of synonymous and cryptic species likely occurs in other eurytomid species treated by Bugbee and are in need of taxonomic revision as many morphological characters used to distinguish Nearctic eurytomids are highly variable. Issues that have long impeded the ecological and biological studies on eurytomids associated with cynipid rose galls such as host specificity and sex association were readily resolved using the integrative taxonomy approach, providing new insights into the evolutionary history of this taxonomically difficult group.

Table 2.1. Primers used for PCR and sequencing

Primer Name	Direction	Primer Sequence (5'-3')	Primer Source
LepF1	Forward	ATTCAACCAATCATAAAGATATTGG	Hebert *et al.* (2004)
FWPTF1	Forward	CCTGGTTCTTTRATTGGTAATGATC	Li *et al.* (2010)
RonMWASPdeg_t1	Forward	TGTAAAACGACGGCCAGTGGWTCW CCWGATATAKCWTTTCC	Smith *et al.* (2008)
UEA3	Forward	TATAGCATTCCCACGAATAAATAA	Lunt *et al.* (1996)
TL2-N-3014	Reverse	TCCATTGCACTAATCTGCCATATTA	Simon *et al.* (1994)
C_ANTMR1D-RonIIdeg_R	Reverse	GGRGGRTARAYAGTTCATCCWGTWCC	Modified from Simon *et al.* (1994)
C_ANTMR1D-AMR1deg_R	Reverse	CAWCCWGTWCCKRMNCCWKCAT	Smith *et al.* (2005)
LepR1	Reverse	TAAACTTCTGGATGTCCAAAAATCA	Hebert *et al.* (2004)

Table 2.2. Species of eurytomids associated with galls of Nearctic *Diplolepis* (Noyes 2012). Canadian records are indicated in bold.

Species	Host	Distribution
Eurytoma acuta		
Bugbee 1951	*D. spinosa, D. tumida, D. variabilis*	**MB**, **SK**, AR, CO, MI, MN, ND, UT
Eurytoma calcarea		
Bugbee 1951	*D. bicolor, D. nebulosa, D. variabilis*	IL, ME, MA, NE, UT
Eurytoma discordans		
Bugbee 1951	*D. nodulosa, D. variabilis*	**AB**, **ON**, **QC**, IN, ME, MA, MI, VA, WI
Eurytoma flavicrurensa		
Bugbee 1951	*D. polita*	OR
Eurytoma hebes		
Bugbee 1973	*D. polita*	**AB**
Eurytoma imminuta		
Bugbee 1951	*D. variabilis*	NV, OR, UT
Eurytoma incerta		
Fullaway 1912	*D. californica, D. polita, D. spinosa*	**SK**, CA, OR
Eurytoma iniquus		
Bugbee 1951	*D. spinosa*	CO
Eurytoma longavena		
Bugbee 1951	*D. bicolor, D. polita*	**AB, BC**
Eurytoma nigricoxa		
Provancher 1887	*Periclistus* sp.	**BC, MB, ON, QC**
Eurytoma obtusilobae		
Ashmead 1885	*D. radicum*	**MB**, FL, IL, MA, MN, NY, OR, TX, UT, VA, CA
Eurytoma spina		
Bugbee 1951	*D. spinosa*	**SK**, OR
Eurytoma spongiosa		
Bugbee 1951	*D. dicholcerus, D. rosae, D. spinosa, D. tumida, D. triforma*	**ON**, **SK**, AR, CO, IL, IN, IA, MD, MA, MI, NY, OH, PA, RI, UT, VA
Eurytoma terrea		
Bugbee 1951	*D. polita*	OR
Tenuipetiolus ruber		
Bugbee 1951	*D. nodulosa, D. rosae*	**ON**, AZ, IL, IN, KS, NY, NC, PA, TN, VA

Table 2.3. Collection locality and host information for eurytomid morphospecies and haplogroups. New records are indicated in bold.

Morphospecies	Haplogroups	Locality	Host Gall	Host Plant	Sample Size (*n*)
Eurytoma acuta	*Eurytoma discordans*	Alberta	*D. nodulosa*	*R. woodsii*	4
		Alberta	*D. tumida*	*R. woodsii*	7
Eurytoma calcarea		Alberta	*D. bicolor*	*R. woodsii*	10
		British Columbia	*D. variabilis*	*R. woodsii*	3
Eurytoma discordans		Ontario	*D. nodulosa*	*R. blanda*	4
		Ontario	***D. radicum***	*R. acicularis*	6
		Ontario	*D. spinosa*	*R. blanda*	20
Eurytoma iniquus	***Eurytoma iniquus***	**Alberta**	***D. bicolor***	*R. woodsii*	2
		British Columbia	***D. variabilis***	*R. woodsii*	1
		Ontario	***D. bicolor***	*R. blanda*	4
		Ontario	***D. nodulosa***	*R. blanda*	3
		Ontario	***D. rosaefolii***	*R. acicularis*	2
Eurytoma longavena	*Eurytoma longavena*	Alberta	***D. nebulosa***	*R. woodsii*	2
		British Columbia	***D. variabilis***	*R. woodsii*	1
		Quebec	*D. polita*	*R. acicularis*	6
		Ontario	*D. nebulosa*	*R. blanda*	6
		Ontario	***D. fusiformans***	*R. acicularis*	2
		Ontario	*D. polita*	*R. acicularis*	4
		Ontario	***D. rosaefolii***	*R. acicularis*	15
Eurytoma spongiosa	*Eurytoma spongiosa* 1	**Alberta**	***D. ignota***	*R. woodsii*	2
		Alberta	*D. tumida*	*R. woodsii*	5
		British Columbia	***D. variabilis***	*R. woodsii*	4
		Ontario	*D. spinosa*	*R. blanda*	15
		Ontario	*D. spinosa*	*R. rugosa*	7
		Ontario	*D. triforma*	*R. canina*	24
	Eurytoma spongiosa 2	Alberta	*D. ignota*	*R. arkasana*	5
		Ontario	***D. nebulosa***	*R. blanda*	1
		Ontario	*D. triforma*	*R. acicularis*	7
		Ontario	*D. triforma*	*R. canina*	19
		Quebec	***D. polita***	*R. acicularis*	4
Eurytoma sp.	***Eurytoma* sp 1**	**Ontario**	***D. nodulosa***	*R. blanda*	1
		Ontario	***D. rosaefolii***	*R. acicularis*	3
	***Eurytoma* sp 2**	**Alberta**	***D. ignota***	*R. arkasana*	1
Tenuipetiolus ruber	*Tenuipetiolus ruber*	Ontario	***D. triforma***	*R. acicularis*	2
		Quebec	***D. polita***	*R. acicularis*	2

Table 2.4. Intra- and inter-specific divergence for all haplogroups. Standard errors in blue are shown in reverse of the matrix for interspecific divergence.

Interspecific Divergence	*Eurytoma discordans*	*Eurytoma iniquus*	*Eurytoma longavena*	*Eurytoma* sp1	*Eurytoma* sp2	*Eurytoma spongiosa* 1	*Eurytoma spongiosa* 2	*Tenuipetiolus ruber*	**Intraspecific Divergence**	**S.E.**
Eurytoma discordans		0.018	0.017	0.016	0.021	0.023	0.014	0.025	0.014	0.005
Eurytoma iniquus	0.092		0.024	0.021	0.023	0.024	0.019	0.028	0.012	0.004
Eurytoma longavena	0.079	0.126		0.018	0.022	0.023	0.018	0.027	0.002	0.002
Eurytoma sp1	0.076	0.117	0.090		0.021	0.022	0.018	0.028	0.028	0.008
Eurytoma sp2	0.097	0.132	0.103	0.112		0.023	0.021	0.029	N/A	N/A
Eurytoma spongiosa 1	0.128	0.134	0.118	0.125	0.120		0.021	0.029	0.004	0.002
Eurytoma spongiosa 2	0.057	0.088	0.076	0.089	0.097	0.103		0.026	0.005	0.002
Tenuipetiolus ruber	0.161	0.197	0.175	0.194	0.202	0.198	0.170		0.038	0.011

Table 2.5. Eurytomid haplogroups and associated rose galls

Species	**Plant Organ**	***Eurytoma discordans***	***Eurytoma iniquus***	***Eurytoma longavena***	***Eurytoma spongiosa* 1**	***Eurytoma spongiosa* 2**	***Eurytoma* sp1**	***Eurytoma* sp2**	***Tenuipetiolus ruber***
D. bicolor	Leaf	**X**	**X**						
D. ignota	Leaf				**X**	**X**		**X**	
D. nebulosa	Leaf			**X**		**X**			
D. polita	Leaf			**X**		**X**			**X**
D. rosaefolii	Leaf		**X**	**X**			**X**		
D. variabilis	Leaf	**X**	**X**	**X**	**X**				
D. fusiformans	Stem			**X**					
D. nodulosa	Stem	**X**	**X**				**X**		
D. spinosa	Stem	**X**			**X**				
D. triforma	Stem				**X**	**X**			**X**
D. tumida	Stem	**X**			**X**				
D. radicum	Root	**X**							

Figure 2.1. Map of Canada indicating the sampling locations of eurytomids.

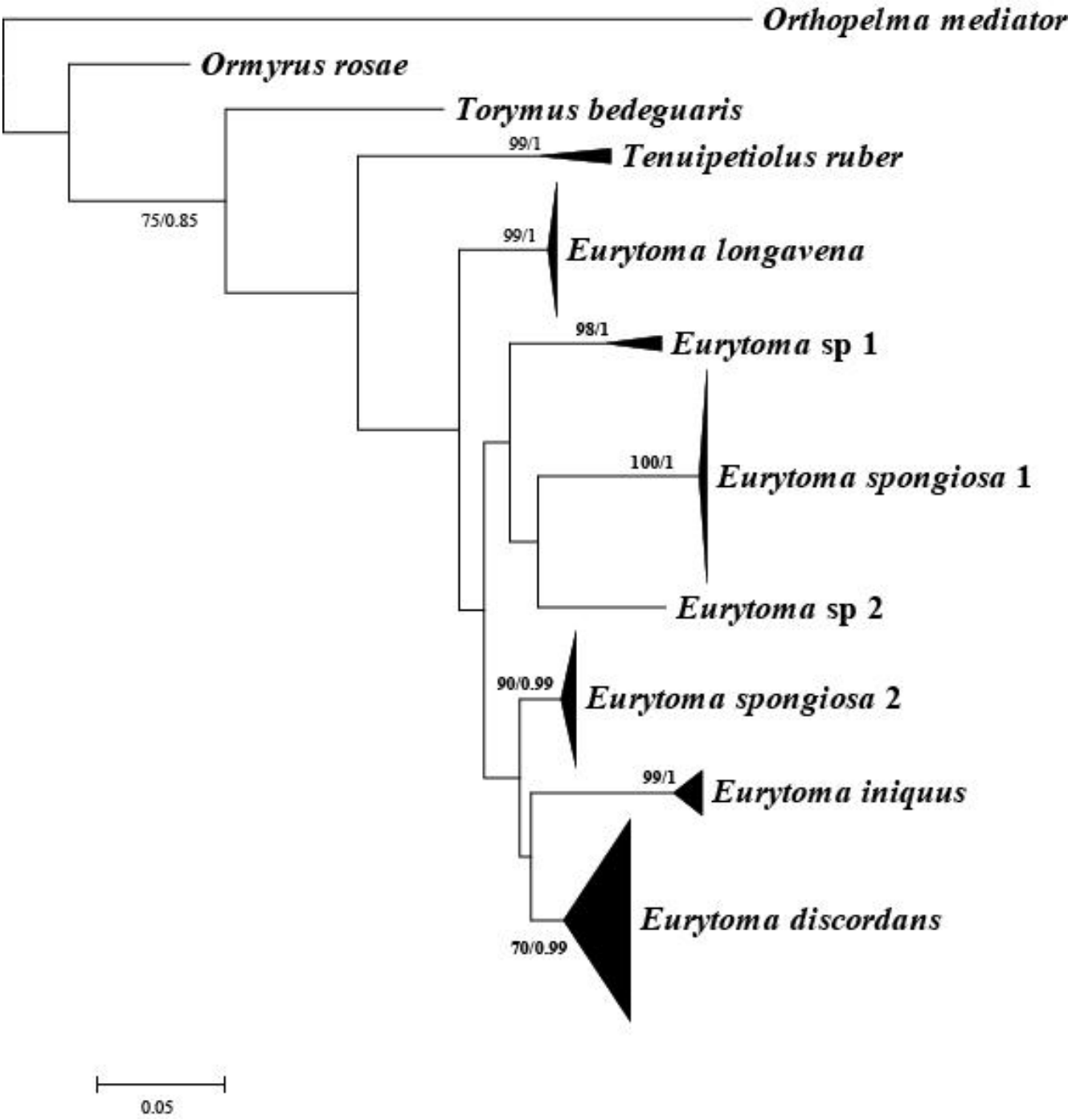

Figure 2.2. Phylogenetic tree for species of eurytomids associated with rose galls induced by *Diplolepis* in Canada based on COI data. Maximum likelihood bootstrap support (first value) and Bayesian posterior probabilities (second value) are shown at each node. The scale bar represents the number of nucleotide substitutions per site. *Orthopelma mediator* (Ichneumonidae), *Ormyrus rosae* (Ormyridae) and *Torymus bedeguaris* (Torymidae) are used as outgroups.

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Chapter 3 - Redescription of Canadian Eurytomidae (Hymenoptera, Chalcidoidea) associated with galls induced by cynipid wasps of the genus *Diplolepis* (Hymenoptera, Cynipidae)

Abstract

Eurytomids are small parasitic wasps associated with many communities of phytophagous insects. In most cases, the accurate identification of eurytomids is impeded by inadequate species descriptions that do not include figures of diagnostic features, and keys that are difficult to use. Here, diagnostic features and redescriptions are provided for both sexes of the eurytomids associated with galls induced by cynipid wasps of the genus *Diplolepis* found on shrub roses across Canada. Consequently six species of *Eurytoma* Illiger, along with *Tenuipetiolus ruber* Bugbee, were found. One new species, with the manuscript name "*Eurytoma spongiosa*2*"* is proposed **sp. nov.**. New synonyms include *Eurytoma hebes* Bugbee under *Eurytoma longavena* Bugbee **n.syn.** , *Eurytoma imminuta* Bugbee under *Eurytoma spongiosa* Bugbee **n.syn.**, and both *Eurytoma acuta* Bugbee and *Eurytoma calcarea* Bugbee under *Eurytoma discordans* Bugbee **n.syn.** Several new host and distribution records are reported, including new Canadian records. A dichotomous key is provided for both sexes of all seven species using photographs and scanning electron microscopy images.

Introduction

The interaction between insect herbivores and their associated natural enemies is one of the key factors in understanding the origins and evolution of multi-trophic systems. One particularly species-rich, ecologically-closed model system for studies of host-parasitoid relationships is the community of cynipids and their associated inquilines and parasitoids (predominantly chalcids of the order Hymenoptera) on oaks and roses (Csóka *et al.* 2005, Nicholls *et al.* 2010). Parasitoids associated with cynipid galls feed either internally or externally as the host continues to develop (koinobionts), or when development of the host has been arrested by stinging prior to oviposition (idiobionts; Csóka *et al.* 2005). All parasitoids that attack cynipid hosts are wasps, including members of Ichneumonidae and families in the superfamily Chalcidoidea. Approximately 200 species of hymenopteran parasitoids are known from cynipid galls in Europe and North America, most of which are gall-specific (Csóka *et al.* 2005).

For instance, parasitoids associated with galls induced by cynipid wasps of the genus *Diplolepis*, in particular the European species *D. rosae* (Stille 1984, Lázsló & Tóthmérész 2006, 2011, Lotfalizadeh *et al.* 2007c) and the North American species *D. polita* and *D. nodulosa* (Brooks & Shorthouse 1997, Shorthouse 1973, 2010) have been well studied. However, the taxonomy and ecology of many of these parasitoids are poorly known due to their small size and morphological conservatism (Lotfalizadeh *et al.* 2007c).

One of the most common parasitoids associated with cynipid galls on roses in Canada are members of the family Eurytomidae (Hymenoptera: Chalcidoidea), often comprising up to 40% of the emergents coming from large samples of galls induced by the same species in various

localities (Shorthouse 2010). Larvae of all eurytomids found in cynipid galls feed as external koinobionts on either the gall inducers, inquiline cynipids of the genus *Periclistus* or other parasitoids (Csóka *et al.* 2005, Shorthouse 2010). Some eurytomids have also been recorded feeding on gall tissues after consuming their insect hosts (Leggo & Shorthouse 2006).

Recent phylogenetic analyses redefined Eurytomidae as a monophyletic group consisting of two subfamilies, Buresiinae and Eurytominae (Lotfalizadeh *et al.* 2007b). The subfamilies Heimbrinae and Rileyinae are excluded from Eurytomidae, however no formal erection of new family names has been made (Lotfalizadeh *et al.* 2007b, Gates 2008). A gradual and mosaic evolution with large levels of homoplasy was observed within Eurytominae based on the study by Lotfalizadeh *et al.* (2007b). The genus *Tenuipetiolus* Bugbee was grouped with *Prodecatoma* Ashmead based on the following derived states: 1) Adscrobal area with a dorsal depression or areola; 2) Epicnemium with a large and circular median areola dorsally; 3) Precoxal carinae close to anterior margin of metapleuron; 4) Submedian carinae close to each other. However no formal synonymization has been proposed and all four species of *Tenuipetiolus* are found restricted within Nearctic, associated with galls of cynipids and cecidomyiids (Bugbee 1951a).

All species with a carinate gena and showing no other outstanding characters were redefined as *Eurytoma sensu stricto* (*s.s.*) with the following derived states: 1) Postgenal lamina present and raised ventrally over the surface of the postgena; 2) Postgena with a ventral depression between the posterior margin of the gena and the hypostomal fossa, with the depression delimited dorsally by a ridge or a step; 3) Gena with posterior margin slightly angulate above oral fossa (Lotfalizadeh *et al.* 2007b). *Eurytoma s.s.* is divided into 11 species groups including 700 nominal species worldwide, with ~100 Nearctic species north of Mexico

(Lotfalizadeh *et al*. 2007b). *Eurytoma* associated with cynipid gall inducers have been placed under the *rosae* group, characterized by the presence of a precoxal tooth formed by the adscrobal carina (Lotfalizadeh *et al*. 2007b). Members of the *rosae* group often include cryptic species, which were morphologically indistinct or similar but with genetic and biological differences (Claridge & Askew 1960, Ács *et al.* 2002, Lotfalizadeh *et al*. 2007a, Gómez *et al.* 2011). A total of nine species of *Eurytoma* Illiger and one species of *Tenuipetiolus* have been recorded associated with cynipid galls on roses in North America (Noyes 2012). However, the most recent published key to Nearctic *Tenuipetiolus* (Bugbee 1951a) and *Eurytoma* (Bugbee 1951b, Bugbee 1967) is difficult to use due to the overlapping character states and the lack of illustrations. Additionally, current identification keys are limited to females, and thus the males are unidentifiable to species level.

The objective of this study is to describe both male and female eurytomids associated with rose galls in Canada, as well as updating morphological characters, hosts, and distributional records. Additionally a dichotomous key is provided for both sex of all seven species.

Materials & Methods

The eurytomids used for this study were from the collection of J. D. Shorthouse at Laurentian University in Sudbury, Ontario, and referred to as JDSC. This collection consists of pin-mounted inhabitants, and those stored in vials of alcohol, that had emerged from thousands of galls that were collected across Canada over the past 45 years. In most cases, the eurytomids, along with all other gall inhabitants, were obtained by storing mature galls in glass jars in the

laboratory and aspirating the insects daily when they appeared. In other cases, the larvae of gall inhabitants were removed from mature galls either in the fall or spring and placed in pin-mounted gelatin capsules. Adults obtained in this manner were cleaner than those that had chewed their way out of galls and could be associated with the larval stage. Most specimens from these collections were chemically dried using hexamethldisilazane (HMDS), following the protocol of Heraty & Hawks (1998) before they were point- or card- mounted. A full list of specimens examined is listed under each redescription. Additional specimens were also borrowed from the following institutions:

CNC Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada, (John T. Huber, Curator).

DEBU University of Guelph Insect Collection, Guelph, Ontario, Canada, (Stephen A. Marshall, Curator).

USNM National Museum of Natural History, Washington D.C., USA, (Michael W. Gates, Curator).

Scanning electron microscopy (SEM) and stereomicroscope photographs were taken using methods described by Gates and Pérez-Lachaud (2012). Composite descriptions were made based on a combination of type specimens with DNA vouchers. Terminologies used for surface sculptures follow Harris (1979), while the morphology follows Gibson (1997), Lotfalizadeh *et al.* (2007b), and Gates & Pérez-Lachaud (2012). Detailed illustrations of the characters are found in Appendix III. The following acronyms are used: **LOL** (Lateral Ocellar

Line), **OOL** (Ocellocular Line), **POL** (Posterior Ocellar Line), $\mathbf{F_n}$ (Funicular segment #), $\mathbf{Gt_n}$ (Gastral tergite #). Measurements were taken using an ocular micrometer, and size range given is based on the largest and smallest specimens in the material examined. Descriptions are based on females, with differences in males listed after. Distribution maps were produced using Simplemappr (Shorthouse 2012, www.Simplemappr.net).

Results

Key to species of Eurytomidae associated with galls induced by *Diplolepis* in Canada

1. Female: Gt_1 and Gt_2 fused dorsally (Fig. 3.31). Petiole longer than metacoxae (Fig. 3.13).
   Male: F3–F5 has one row of setae. Petiole 2x longer than metacoxae

   ….……..……..….***Tenuipetiolus ruber*** Bugbee

1'. Female: Gt_1 and Gt_2 not fused dorsally (Fig. 3.29). Petiole subequal to metacoxae in length.
   Male: F3–F5 has 2–3 rows of setae. Petiole <2x longer than metacoxae in length

…….……...……..……. ***Eurytoma*** Illiger; 2

2. Tegulae, scape and pedicle yellow (Fig. 3.11, 3.12).……...……..***E. obtusilobae*** Ashmead

2'. Tegulae black to brown, scape and pedicle with black with yellow restricted to basal region (Fig. 3.1, 3.2)…….……..…3

3. Fore- and midcoxae yellow to brown, never black (Fig. 3.3, 3.4)……..***E. iniquus*** Bugbee

3'. Fore- and midcoxae entirely black (Fig. 3.1, 3.2).……...4

4. Female: Gaster S-curve shaped (Fig. 3.1, 3.29), longer than head + mesosoma, ovipositor sheath upturned (Fig. 3.1). Male: Funicles longer than wide, with 2 rows of longitudinal sensilla and adpressed setae (Fig. 3.39)……..**.....*E. discordans*** Bugbee

4'. Female: Gaster not S-curve shaped (Fig. 3.5, 3.30), shorter or equal to head + mesosoma, ovipositor sheath not upturned. Male: Funicles as long as wide, with 1 row of longitudinal sensilla and erect setae (Fig. 3.40)……...…5

5. Scape entirely black (Fig. 3.5, 3.6). Forewing marginal vein longer than postmarginal vein (Fig 3.42). Female: Gt_4 large and covers most of Gt_5 (Fig. 3.30)

……..…...…***E. longavena*** Bugbee

5'. Scape black with yellow at extreme base (Fig. 3.8, 3.10). Forewing marginal vein subequal to postmarginal vein. Female: Gt_4 small and does not cover most of Gt_5 (Fig. 3.7, 3.9) …….....6

6. Pro- and mesocoxae yellow, with black limited to proximal regions (Figs. 3.7, 3.8)……...……...…….***E. spongiosa*** Bugbee

6'. Pro- and mesocoxae infuscated (Figs. 3.9, 3.10).....***E. spongiosa2***

***Eurytoma discordans* Bugbee**

(Figs. 3.1, 3.2, 3.15, 3.17, 3.19, 3.22, 3.26, 3.29, 3.32–3.35, 3.37, 3.39)

Eurytoma discordans Bugbee 1951b: 220–223. Holotype ♀ (USNM). Type data: USA, Indiana

[Howe]; associated with galls induced by *Diplolepis globuloides* (Beutenmuller) = (*Diplolepis variabilis* (Bassett)) on species of *Rosa*, Dec 20, 1930.

Eurytoma acuta Bugbee 1951b: 223–234. Holotype ♀ (USNM). Type data: USA, Utah [Price]; associated with galls induced by *Diplolepis tuberculatrix xerophila* (Cockerell) on *Rosa*, April 20, 1920. **N. syn.**

Eurytoma calcarea Bugbee 1951b: 240–249. Holotype ♀ (USNM). Type data: USA, Utah [Wellsville]; associated with galls induced by *Diplolepis variabilis* (Bassett), September 6, 1927. **N. syn.**

Diagnosis Females are distinguished from other eurytomid species by the S-curved metasoma that is larger than head + mesosoma (Fig. 3.29) and the sharply upturned ovipositor dorsad the horizontal axis of the metasoma. Males have elongated funicular segments and yellow fore- and midlegs similar to *E. obtusilobae*, however it differs in the black pedicle and tegulae.

Females: Body Length 2.1–5.0mm. Colour: Black except for the following yellow – basal half of scape, pro- and mesofemora, basal pro- and mesotibiae, apex of metatibiae, tip of ovipositor sheath, tarsomeres 1–4, wing veins (Figs. 3.1, 3.2).

Head: 1.2X as broad as high, umbilicate punctured (Fig 3.15). Genal carina present; malar space 0.7X eye height, clypeus bilobate and supraclypeal area smooth (Fig 3.17). Toruli positioned slightly above lower ocular line. Funicular segments subequal in size, longer than wide; pedicel chalice-shaped; funicle fusiform; F1 slightly narrowed basally, funicular segments with 2 rows of longitudinal sensilla and 3 whorls of setae (Fig. 3.35). Ratio of LOL: OOL: POL is 1: 1.3: 2.

Head posteriorly with postgenal lamina and postgenal grooves ridged, delimited ventrally by postgenal depression. Postgena evenly setose (Fig. 3.19).

Mesosoma: Umbilicate, 1.5X as long as broad; notauli complete, shallow. Epicnemium imbricate, flattened, with superficial submedial, shallow depressions to receive forecoxae. Mesepisternum anterior to femoral depression umbilicate; mesepimeron reticulate ventrally, dorsally delimited from femoral depression by fine carina with femoral depression reticulate (Fig. 3.22). Lateral panels of propodeum and callus umbilicately punctate, distinctly delimited from median area by carinae forming irregular setose cells, median furrow delimited, forming 2 rows of irregular foveae (Fig. 3.26). Procoxa imbricate except anterior surface smooth, superficially depressed anteriorly for reception of lower head (Fig 3.32). Mesocoxa lacking lamella (Fig 3.33). Metacoxa densely setose along anterior margins, glabrate with one row of setae along posterior distal margin (Fig. 3.34). Forewing hyaline, marginal vein and postmarginal vein subequal in length.

Metasoma: Gaster 1.8X as long as mesosoma in lateral view; smooth, anterior edge of gastral tergites microreticulate (Fig. 3.29). Petiole 0.7X as long as broad in dorsal view, with projecting lateral teeth as well as mediodorsal prong. Gaster laterally compressed, S-curve shaped and not convex in lateral view, ovipositor upturned dorsad horizontal axis. $Gt_{1–3}$ glabrate, Gt_4 with 1–4 setae lateromedially, $Gt_{5–8}$ and apex of ovipositor sheath densely setose.

Male: Body length: 1.7–3.1mm. Colour: Black, yellow areas as described for female. Sculpture as described for female. Antennae with funicular segments longer than wide, pedicellate, F2–F5 each with 2 or more rows of apressed setae and 2 rows of longitudinal sensilla (Fig. 3.39); scape with ventral plaque in apical half. Gastral petiole in lateral view cylindrical, in dorsal view length

about 1.5X as long as greatest width, 0.7X times length of metacoxa; evenly reticulate dorsally and ventrally, obliterated laterally (Fig. 3.37).

Remarks: This is a widespread and morphologically variable species. Bugbee (1951b) originally divided this species into 3 based on subtle morphological differences and distribution: the Eastern populations as *E. discordans* and western populations as *E. acuta* (5 subspecies), and those smaller in size as *E. calcarea* (6 subspecies). Upon examining additional materials it was noted that the degree of infuscation on legs and scape are variable within this species and thus cannot be used as reliable distinguishing characters. The molecular results presented in Chapter 2 also support the monophyly of this group, albeit with the highest intra-specific divergence compared to other eurytomids. Considering there are no distinct geographical or host differences that support three distinct species, the species *E. acuta* and *E. calcarea* along with their associated subspecies are synonymized under *E. discordans* pending further molecular or ecological studies.

Biology: Reared from field populations of galls induced by *Diplolepis bicolor, D. nodulosa, D. spinosa, D. radicum, D. tumida, D. variabilis* on *Rosa* spp., and *Diastrophus nebulosus* on *Rubus* spp.

Material Examined (197♀, 120♂): ALBERTA: Beaverlodge, 1933 (1♀, CNC); Head-Smashed-In Buffalo Jump, 10.V.2011, J.D. & M.R. Shorthouse, ex. *Diplolepis tumida* on *Rosa woodsii* (3♀, 4♂, JDSC); Edmonton, 20.IV.1942, R.W.Salt (1♀, CNC); Edmonton, 6.VI.1946, R.M.Mason, ex. rose gall (5♀, CNC); Waterton Lakes National Park, 9.V.2007, J.D. & M.R. Shorthouse, ex. *Diplolepis bicolor/Diplolepis nodulosa* modified by *Periclistus* sp. on *Rosa woodsii* (24♀, 20♂, JDSC). **BRITISH COLUMBIA**: Kelowna airport, 20.V.2008, R.G.

Lalonde, ex. *Diplolepis variabilis* on *Rosa woodsii* (2♀, 1♂, JDSC); Summerland, 2.V.1959, R.E. Leech, exit *Rosa* (3♀, CNC); Surrey, 9.IV.1954. K. Yamanaka, ex. *Rubus* (4♀, 2♂, CNC). **MANITOBA**: Morden, 1.IX.1986, J.D. Shorthouse, ex. *Rosa woodsii* (7♀, 3♂, JDSC); Sandlands F.R., em 1.VI.1944, F.I.Survey, stem gall on raspberry (2♀, CNC). **NEW BRUNSWICK**: Kouchibouguac National Park, 8.VIII.1977, S.J. Miller (3♀, 9♂, CNC). **ONTARIO**: Attawapiskat, 18.V.2005, M.J.T. Bodnar, ex. *Diplolepis spinosa* on *Rosa blanda* (22♀, 20♂, JDSC); Bell's Corners, 13.V.1940. O. Peck, Host *Diastrophus nebulosus* (1♀, CNC); Chelmsford, 5.V.1994, S.E. Brooks, ex. *Periclistus pirata*-modified galls of *Diplolepis nodulosa* (4♀, 2♂, JDSC); Cochrane, 24.IV.2010, J.D. Shorthouse & Y.M. Zhang, ex. *Diplolepis spinosa* on *Rosa blanda* (5♀, 5♂, JDSC); Fort Albany, 28.V.2005, M.J.T. Bodnar, ex. *Diplolepis radicum* on *Rosa acicularis* (4♀, 2♂, JDSC); Jockvale, 8–27.V.1955, O. Peck, *Rosa blanda*/*rugosa* (42♀, 19♂, CNC); Manitoulin Island, 29.IV.2011, J.D. Shorthouse, B.L. Smallwood & Y. M. Zhang, ex. *Diplolepis nodulosa* modified by *Periclistus* sp. (2♀, 1♂, JDSC); Marmora, 20.VI.1945. G.R.Hammond. Cynipid gall on wild rose (4♀, CNC); Merivale, 17.VI.1954. O. Peck, Host gall *Periclistus pirata* ex *Rosa blanda* (3♀, 1♂, CNC); Moose Factory Island, 23.IV.2010, J.D. Shorthouse & Y.M. Zhang, ex. *Diplolepis spinosa* on *Rosa blanda* (5♀, 5♂, JDSC); One Sided Lake, em XII.1960. S.M. Clark (2♀, CNC); Ottawa, 17–25.V.1955, O. Peck, ex. *Rosa rugosa* (19♀, 4♂, CNC); Thamesville, 4.VII.1962. S.M.Clark, ex gall of wild rose (3♀, CNC); Rockcliffe, 27.V.1959. S.M.Clark. gall on *Rosa* sp. (3♂, CNC). **PRINCE EDWARD ISLAND**: Eldon, J.D. & M.R. Shorthouse, 23.VIII.1992, ex. *Periclistus pirata*-modified galls of *Diplolepis nodulosa* on *Rosa virginiana* (4♀, 3♂, JDSC). **QUÉBEC**: Aylmer, VIII.1939. E.G. Lester, Host *Periclistus sylvestris* (2♀, 5♂, CNC); Chrysostome, 23.VI.1986, Ex. Cynipidae on *Rosa* sp. (1♀, CNC); Lac Mercier, 7.VIII.1937. G.S. Walley. (1♀,

CNC); Montréal, O. Peck, 22.V–11.VI.1962. Ex. *D. radicum* (6♀, 2♂, CNC).

SASKATCHEWAN: Caron, em 20–23.II.1951, F.I.Survey, rose gall (1♀, 3♂, CNC); Great Sand Hills, 22.IX.1999, J.D. & M.R. Shorthouse, ex. *Diplolepis radicum* on *Rosa woodsii* (6♀, 6♂, JDSC); Snowden, 18.VII.1944, O. Peck (1♀, CNC); White Fox, 17.VI.1944, O. Peck (3♀, CNC).

Distribution: Widespread, from Alberta, British Columbia, Manitoba, New Brunswick, Ontario, Prince Edward Island, Québec, and Saskatchewan (Fig. 3.43).

***Eurytoma iniquus* Bugbee**

(Figs. 3.3, 3.4)

Eurytoma iniquus Bugbee 1951b: 253–254. Holotype ♀ (USNM). Type data: USA, Colorado [Manitou]; associated with galls induced by *Diplolepis neglectus* (Gillette) on species of *Rosa*, April 24, 1920.

Diagnosis: This species is similar to *E. discordans*, it can be distinguished by the yellow infuscation on the inner face of pro- and mesocoxae, whereas other species all have coxae that are entirely black.

Females: Body length 2.2–3.0 mm. Colour: Black except for the following yellow to brown: inner faces of forecoxae, fore- and midfemora and tibiae, apices of hind legs, protibiae laterally, tip of ovipositor sheath, tarsomeres 1-4, wing veins (Figs. 3.3, 3.4).

Head: Head 1.3X as broad as high, umbilicate punctured with small tentorial pits. Genal carina present; malar space 0.8X eye height, clypeus bilobate and supraclypeal area smooth. Toruli positioned about half way above lower ocular line. Antennae with funicular segments subequal in size; pedicel chalice-shaped; funicle fusiform; F1 slightly narrowed basally, funicular segments with 2 rows of longitudinal sensilla and 3 whorls of setae; clava bisegmented. Ratio of LOL: OOL: POL is 1: 1: 2.

Mesosoma: Umbilicate, 1.4X as long as broad; notauli complete, shallow. Epicnemium imbricate, flattened, with superficial submedial, shallow depressions to receive forecoxae. Mesepisternum anterior to femoral depression umbilicate; mesepimeron reticulate ventrally, dorsally delimited from femoral depression by fine carina with femoral depression reticulate. Lateral panels of propodeum and callus umbilicately punctate, distinctly delimited from median area by carinae forming irregular setose cells, median furrow delimited, forming 2 rows of irregular asetose cells. Procoxa imbricate except anterior surface smooth, superficially depress anteriorly for reception of lower head. Mesocoxa lacking lamella. Metacoxa sparsely setose anteriorly and one row of setae on the posterior apical margin. Forewing hyaline, marginal vein subequal to postmarginal vein in length.

Metasoma: Gaster 1.8X as long as mesosoma in lateral view; smooth, anterior edge of gastral tergites microreticulate. Petiole 0.5X as long as broad in dorsal view, with projecting lateral teeth as well as mediodorsal prong. Gaster laterally compressed, oval shaped and convex in lateral view, ovipositor slightly upturned dorsad the horizontal axis. $Gt_{1–4}$ glabrate, $Gt_{5–8}$ and apex of ovipositor sheath setose.

Male: Body length: 1.7–2.8mm. Colour: Black, yellow areas as described for female. Sculpture as described for female. Antennae with funicular segments pedicellate, F2–F5 each with 2 rows of erect setae and 1 row of longitudinal sensilla; scape with ventral plaque in apical half. Gastral petiole in lateral view cylindrical, in dorsal view length about 1.8X as long as greatest width, 0.6X times length of metacoxa; evenly reticulate dorsally and ventrally, obliterated laterally.

Remarks: This species is likely a parasitoid of the inquiline *Periclistus* rather than the gall inducer, as they are reared from hosts that have a high rate of inquilism (See Chapter 2). *Eurytoma iniquus* is particularly abundant in *Periclistus*-modified galls induced by *D. nodulosa,* which are morphologically distinct from unmodified galls. As the inducer larvae are killed during oviposition by the inquiline, the only inhabitants that are abundant within these galls are inquiline larvae. *E. iniquus* are morphologically distinct to the holotype of *Eurytoma nigricoxa* Provancher, which has all coxae yellow. *E. nigricoxa* is the only Nearctic species recorded in association with *Periclistus*, however this likely an error as the holotype lacks any biological information. Whereas other specimens from CNCI are *E. iniquus* that have been misidentified as *E. nigricoxa* (Bugbee 1951b).

Biology: Reared from field populations of galls induced by *Diplolepis bicolor, D. nodulosa, D. polita, D. rosaefolii, D. variabilis* on *Rosa* spp.

Material Examined (33♀, 20♂): ALBERTA: Peace River, 16.VIII.1970, J.D. & M.R. Shorthouse, ex. *Diplolepis polita* fall/spring emergence ((4♀, 2♂, JDSC); Waterton Lakes National Park, 11.V.2011, J.D. & M.R. Shorthouse, ex. *Diplolepis biocolor* on *Rosa blanda* (2♀, JDSC). **BRITISH COLUMBIA**: Kelowna, 20.V.2008, R.G. Lalonde, ex. *Diplolepis variabilis* on *Rosa woodsii* (1♂, JDSC). **ONTARIO**: Chelmsford, 5.V.1994, S.E. Brooks, ex. *Periclistus*

pirata-modified galls of *Diplolepis nodulosa* (6♀, JDSC); Manitoulin Island, 29.IV.2011, J.D. Shorthouse, B.L. Smallwood & Y.M. Zhang, ex. *Diplolepis nodulosa* modified by *Periclistus* sp. (4♀, 2♂, JDSC); Red Lake, 18.V.2002, J.D. Shorthouse & S.T. Offman, ex. *Diplolepis rosaefolii* on *Rosa acicularis* (2♂, JDSC); Thunder Bay, 15.V.2002, J.D. Shorthouse & S.T. Offman, ex. *Diplolepis bicolor* on *Rosa blanda* (14♀, 10♂, JDSC). **PRINCE EDWARD ISLAND**: Eldon, J.D. & M.R. Shorthouse, 23.VIII.1992, ex. *Periclistus pirata*-modified galls of *Diplolepis nodulosa* on *Rosa virginiana* (3♀, 3♂, JDSC).

Distribution: From Ontario, Prince Edward Island, and Québec (Fig. 3.44).

***Eurytoma longavena* Bugbee**

(Figs. 3.5, 3.6, 3.20, 3.23, 3.24, 3.30, 3.36, 3.40, 3.42)

Eurytoma longavena Bugbee 1951b: 249–250. Holotype ♀ (USNM). Type data: CANADA, British Columbia [Terrance]; associated with galls induced by *Diplolepis bicolor* (Ashmead) on species of *Rosa*, 1927.

Eurytoma hebes Bugbee 1973: 13–14. Holotype ♀ (USNM). Type data: CANADA, Alberta [Peace River]; associated with galls induced by *Diplolepis polita* (Ashmead) on species of *Rosa*, August 16, 1970. **N. syn.**

Diagnosis: This species differs from other eurytomids in the wholly black scape, legs (except apices of femora and tibiae), and the marginal veins are longer than the postmarginal veins. Additionally the females have large Gt_4 that covers most of Gt_5.

Females: Body length 3.0–3.7mm. Colour: Black, except for the following yellow to brown: apices of all legs, protibiae laterally, tip of ovipositor sheath, tarsomeres, wing veins (Figs. 3.5, 3.6).

Head: Head 1.25X as broad as high, umbilicate punctured with small tentorial pits. Genal carina present; malar space 0.8X eye height, clypeus emarginate and supraclypeal area smooth. Toruli positioned about submedial to lower ocular line. Funicular segments subequal in size; pedicel chalice-shaped; funicle fusiform; F1 slightly narrowed basally, funicular segments with 2 rows of longitudinal sensilla and 3 whorls of setae (Fig. 3.46); Ratio of LOL: OOL: POL is 1: 1.6: 2.5. Head posteriorly with postgenal lamina and postgenal grooves ridged, delimited ventrally by postgenal depression. Postgena sparsely setose (Fig. 3.20).

Mesosoma: Umbilicate, 1.2X as long as broad; notauli complete, shallow (Fig. 3.23). Epicnemium imbricate, flattened, with superficial submedial, shallow depressions to receive forecoxae (Fig. 3.24). Mesepisternum anterior to femoral depression umbilicate; mesepimeron reticulate ventrally, dorsally delimited from femoral depression by fine carina with femoral depression reticulate. Lateral panels of propodeum and callus umbilicately punctate, distinctly delimited from median area by carinae forming irregular setose cells, median furrow delimited, forming 2 rows of irregular asetose cells. Procoxa imbricate except anterior surface smooth, superficially depress anteriorly for reception of lower head. Mesocoxa lacking lamella. Metacoxa sparsely setose anteriorly and one row of setae on the posterior apical margin. Forewing hyaline, marginal vein distinctly longer than postmarginal vein in length (Fig 3.42).

Metasoma: Gaster 1.3X as long as mesosoma in lateral view; smooth, anterior edge of gastral tergites microreticulate (Fig. 3.30). Petiole 0.6X as long as broad in dorsal view, with projecting lateral teeth as well as mediodorsal prong. Gaster laterally compressed, oval shaped and convex in lateral view, ovipositor parallel to horizontal axis. $Gt_{1–4}$ glabrate, $Gt_{5–8}$ and apex of ovipositor sheath setose. Gt_4 emarginate to expose Gt_5 spiracle.

Male: Body length: 1.7–2.2mm. Colour: Black, yellow areas as described for female. Sculpture as described for female. Antennae with funicular segments pedicellate, F2–F5 each with 2 rows of erect setae and 1 row of longitudinal sensilla (Fig. 3.40); scape with ventral plaque in apical half. Gastral petiole in lateral view cylindrical, in dorsal view length about 1.5X as long as greatest width, 0.6X times length of metacoxa; evenly reticulate dorsally and ventrally, obliterated laterally.

Remarks: This widespread species is found from galls of all 7 native species of *Diplolepis* that induce galls on leaves. Additionally, it is collected from stem galls of *D. fusiformans*, a species that is closely related to *D. rosaefolii*, which induces galls on leaves (Plantard *et al.* 1998). Two generations of *E. longavena* have been recorded: "fall emergents" exit spring-induced galls (e.g. *D. polita*) as early as mid-summer to early fall, while "spring emergents" overwinter and exit from galls the following year (Shorthouse 2010). It is likely that fall emergents attack late-summer induced galls (e.g. *D. nebulosa*), however it is unknown what factors determine this bivoltism. The species *E. hebes* shares all the distinguishing characters of *E. longavena* but are smaller in size, thus they are synonymized under the latter.

Biology: Reared from field populations of galls induced by *Diplolepis bassetti, D. bicolor, D. fusiformans, D. gracilis, D. nebulosa, D. polita, D. rosaefolii, and D. variabilis* on *Rosa* spp.

Material Examined (52♀, 22♂): ALBERTA: Coaldale, 24.X.2002, J.D. Shorthouse, ex. *Diplolepis nebulosa* on *Rosa woodsii* (2♀, 1♂, JDSC); Peace River, 16.VIII.1970, J.D. & M.R. Shorthouse, ex. *Diplolepis polita* fall/spring emergence (19♀, 3♂, JDSC). **BRITISH COLUMBIA**: Kelowna, 19.X.1999, R.G. Lalonde, ex. *Diplolepis rosaefolii* on *Rosa woodsii* (7♀, 3♂, JDSC). **ONTARIO**: Cochrane, 24.IV.2010, J.D. Shorthouse & Y.M. Zhang, ex. *Diplolepis rosaefolii* on *Rosa acicularis* (8♀, 7♂, JDSC); Chelmsford, 1.X.1995, J.D.Shorthouse (2♀, CNC); Manitoulin Island, 4.IX. 2010, J.D. & M.R. Shorthouse, ex. *D. nebulosa* on *Rosa blanda* (2♀, 4♂, JDSC); Moose Factory Island, J.D. Shorthouse & M.G. St. John, ex. *Diplolepis polita* on *Rosa acicularis* (4♀, JDSC); Renfrew, 15.IV.2000, J.D. Shorthouse, ex. *Diplolepis fusiformans* on *Rosa blanda* (2♂, JDSC). **QUÉBEC**: La Sarre, B.L. Smallwood & Y.M. Zhang, 13.VII.2010, ex. *D. polita* on *R. acicularis* (5♀, 1♂, JDSC). **SASKATCHEWAN**: Douglas Provincial Park, J.D. & M.R. Shorthouse, 26.IX.1999, ex. *D. gracilis* on *Rosa woodsii* (2♀, 1♂, JDSC); Pike Lake Provincial Park, J.D. & M.R. Shorthouse, 29.IX.1999, ex. *D. gracilis* on *Rosa woodsii* (1♀, JDSC).

Distribution: Widespread, from Alberta, British Columbia, Ontario, Québec, and Saskatchewan (Fig. 3.45).

***Eurytoma obtusilobae* Ashmead**

(Figs. 3.11, 3.12, 3.18, 3.27)

Eurytoma obtusilobae Ashmead 1885: 12. Lectotype ♀ (USNM). Type data: USA, Florida [Jacksonville].

Diagnosis: This species differs from other eurytomids studied in the yellow or brown scape and tegulae, with supraclypeal area strigose.

Females: Body length 3.0–3.9 mm. Colour: Black except for the following yellow to brown: Scape and pedicle, tegulae, fore- and midlegs, apices of hindlegs, tip of ovipositor sheath, tarsomeres 1-4, wing veins (Figs. 3. 11, 3.12).

Head: Head 1.3X as broad as high, umbilicate punctured. Genal carina present; malar space 0.6X eye height, clypeus emarginate and supraclypeal area strigose (Fig 3.18). Toruli positioned about submedial to lower ocular line. Funicular segments subequal in size; pedicel chalice-shaped; funicle fusiform; funicular segments with 2 rows of longitudinal sensilla and 3 whorls of setae. Ratio of LOL: OOL: POL is 1: 1: 1.8. Head posteriorly with postgenal lamina and postgenal grooves ridged, delimited ventrally by postgenal depression. Postgena sparsely setose.

Mesosoma: Umbilicate, 1.2X as long as broad; notauli complete, shallow. Epicnemium imbricate, flattened, with superficial submedial, shallow depressions to receive forecoxae. Mesepisternum anterior to femoral depression umbilicate; mesepimeron reticulate ventrally, dorsally delimited from femoral depression by fine carina with femoral depression reticulate. Lateral panels of propodeum and callus umbilicately punctate, distinctly delimited from median area by carinae forming irregular setose cells, median furrow delimited, forming 2 rows of irregular asetose cells (Fig 3.27). Procoxa imbricate except anterior surface smooth, superficially depress anteriorly for reception of lower head. Mesocoxa lacking lamella. Metacoxa sparsely setose anteriorly and one row of setae on the posterior apical margin. Forewing hyaline, marginal vein subequal to postmarginal vein in length.

Metasoma: Gaster 1.4X as long as mesosoma in lateral view; Smooth, anterior edge of gastral tergites microreticulate. Petiole 0.7X as long as broad in dorsal view, with projecting lateral teeth as well as mediodorsal prong. Gaster laterally compressed, oval shaped and convex in lateral view, ovipositor parallel to horizontal axis. $Gt_{1–4}$ glabrate, $Gt_{5–8}$ and apex of ovipositor sheath setose.

Male: Body length: 2.9–3.6mm. Colour: Black, yellow areas as described for female (Fig. 3.12). Sculpture as described for female. Antenna with funicular segments longer than wide, pedicellate, F2–F5 each with 3 rows of apressed setae and 2 rows of longitudinal sensilla; scape with ventral plaque in apical half. Gastral petiole in lateral view cylindrical, in dorsal view length about 1.4X as long as greatest width, 0.8X times length of metacoxa; evenly reticulate dorsally and ventrally, obliterated laterally.

Remarks: While only found at two sites in this study, the distribution of this species is likely much wider in Canada as *E. obtusilobae* has been broadly collected across USA (Bugbee 1951b). This is likely due to the difficulty in locating their host galls induced by *D. radicum*, which are often covered by soil.

Biology: Reared from field populations of galls induced by *Diplolepis radicum* on *Rosa* spp.

Material Examined (5♀, 3♂): BRITISH COLUMBIA: Kelowna, 14.X.1999, J.D. Shorthouse & R.G. Lalonde, ex. *Diplolepis radicum* on Rosa woodsii (3♀, 3♂, JDSC). **MANITOBA**: Sandilands, 15.V.1967, J.C. Melvin, host gall on rose (2♀).

Distribution: Collected in British Columbia and Manitoba (Fig. 3.46).

***Eurytoma spongiosa* Bugbee**

(Figs. 3.7, 3.8)

Eurytoma spongiosa Bugbee 1951b: 254–258. Holotype ♀ (USNM). Type data: USA, Indiana [Bloomington]; associated with galls induced by *Diplolepis rosae* (L.) on species of *Rosa*, April 14, 1933.

Eurytoma imminuta Bugbee 1951b: 259–260. Holotype ♀ (USNM). Type data: USA, Nevada [Pyramid Lake]; associated with galls induced by *Diplolepis variabilis* (Bassett) on *Rosa puberulenta*, May 20, 1929. **N. syn.**

Diagnosis: Both species of *E. spongiosa* can be distinguished from most *Euryoma* species by their small, oval gaster that is not laterally compressed, black tegulae and scape (Fig. 3.7–3.10). *E. spongiosa* differs from *E. spongiosa*2 in that the black infuscation on the forefemora is limited to the proximal half (Fig. 3.7, 3.8).

Females: Body length 1.9–4.0mm. Colour: Black except for the following yellow – basal half of scape, apical 5th of pro- and mesofemora, basal pro- and mesotibiae, apex of hindlegs, tip of ovipositor sheath, tarsomeres 1–4, wing veins (Figs. 3.7, 3.8).

Head: Head 1.2X as broad as high, umbilicate punctured with small tentorial pits. Genal carina present; malar space 0.8X eye height, supraclypeal area smooth. Toruli positioned slightly above lower ocular line. Funicular segments subequal in size; pedicel chalice-shaped; funicle fusiform; F1 slightly narrowed basally, funicular segments with 2 rows of longitudinal sensilla and 3 whorls of setae; clava bisegmented. Ratio of LOL: OOL: POL is 1: 1.2: 2.4. Head posteriorly

with postgenal lamina and postgenal grooves ridged, delimited ventrally by postgenal depression. Postgena sparsely setose.

Mesosoma: Umbilicate, 1.5X as long as broad; notauli complete, shallow. Epicnemium imbricate, flattened, with superficial submedial, shallow depressions to receive forecoxae. Mesepisternum anterior to femoral depression umbilicate; mesepimeron reticulate ventrally, dorsally delimited from femoral depression by fine carina with femoral depression reticulate. Lateral panels of propodeum and callus umbilicately punctate, distinctly delimited from median area by carinae forming irregular setose cells, median furrow delimited, forming 2 rows of irregular asetose cells. Procoxa imbricate except anterior surface smooth, superficially depress anteriorly for reception of lower head. Mesocoxa lacking lamella. Metacoxa densely setose along anterior margins, glabrate with one row of setae along posterior distal margin. Forewing hyaline, marginal vein and postmarginal vein subequal in length.

Metasoma: Gaster 1.2X as long as mesosoma in lateral view; smooth, anterior edge of gastral tergites microreticulate. Petiole 0.5X as long as broad in dorsal view, with projecting lateral teeth as well as mediodorsal prong. Gaster not laterally compressed, oval shaped and convex in lateral view, ovipositor parallel to horizontal axis. $Gt_{1–3}$ glabrate, Gt_4 with 1–4 setae lateromedially, $Gt_{5–8}$ and apex of ovipositor sheath densely setose.

Male: Body length: 1.7–2.0mm. Colour: Black, yellow areas as described for female. Sculpture as described for female. Antennae with funicular segments pedicellate, F2–F5 each with 2 rows of erect setae and 1 row of longitudinal sensilla; scape with ventral plaque in apical half. Gastral petiole in lateral view cylindrical, in dorsal view length about 1.5X as long as greatest width, 0.5X times length of metacoxa; irregularly reticulate dorsally and ventrally, obliterated laterally.

Remarks: This species includes *E. imminuta*, which is restricted to British Columbia and Alberta attacking galls induced by *D. variabilis* and *D. ignota*. The metasoma of *E. imminuta* is slightly shorter than that of *E. spongiosa*, due to low % divergence between the two species (see Chapter 2) they have been synonymized under the more senior name *E. spongiosa*.

Biology: Reared from field populations of galls induced by *Diplolepis ignota, D. nebulosa, D. polita, D. spinosa, D. triforma, D. tumida,* and *D. variabilis* on *Rosa* spp.

Material Examined (101♀, 50♂): ALBERTA: Coaldale, 12.V.2007, J.D. & M.R. Shorthouse, ex. *Diplolepis ignota* on *Rosa woodsii* (2♀, JDSC); Head-Smashed-In Buffalo Jump, 10.V.2011, J.D. & M.R. Shorthouse, ex. *Diplolepis tumida* on *Rosa woodsii* (3♀, 2♂, JDSC). **BRITISH COLUMBIA:** Kelowna, 20.V.2008, R.G. Lalonde, ex. *Diplolepis variabilis* on *Rosa woodsii* (3♀, 1♂, JDSC). **ONTARIO**: Cochrane, 24.IV.2010, J.D. Shorthouse & Y.M. Zhang, ex. *Diplolepis spinosa* on *Rosa blanda*, (5♀, JDSC); Kanata, 19.IV.2006, J.D. & M.R. Shorthouse, ex. *Diplolepis spinosa* on *Rosa rugosa* (2♀, 6♂, JDSC); Manitoulin Island, 2.V.2009, J.D. Shorthouse & J.D. Renelli, ex. *Diplolepis triforma* on *Rosa canina* (7♀, 3♂, JDSC); Manitoulin Island, 3.V.2009, J.D. Shorthouse & J.D. Renelli, ex. *Diplolepis rosae* on *Rosa canina* (5♀, 1♂, JDSC); Manitoulin Island, 6.V.2010, J.D. Shorthouse & Y.M. Zhang, ex. *Diplolepis triforma* on *Rosa canina* (9♀, 5♂, JDSC); Moose Factory Island, 23.IV.2010, J.D. Shorthouse & Y.M. Zhang, ex. *Diplolepis spinosa* on *Rosa blanda*, (5♀, 5♂, JDSC); Picton, 28.IV.2003, J.D. Shorthouse, ex. *Diplolepis mayri/D. rosae* on *Rosa canina* (12♀, 7♂, JDSC). **MANITOBA**: Mordon, 1.IX.1986, J.D. Shorthouse, ex. *Diplolepis spinosa* on *Rosa woodsii* (31♀, 18♂, JDSC). **QUÉBEC**: Mt. Tremblant, 23.XII.2004, M.J.T. Bodnar, ex. *Diplolepis spinosa* on *Rosa rugosa* (17♀, 2♂, JDSC).

Distribution: Widespread, from Alberta, British Columbia, Ontario, Manitoba, and Québec (Fig. 3.47).

***"Eurytoma spongiosa*2" sp. nov.**

(Figs. 3.9, 3.10, 3.38, 3.41)

Diagnosis: Similar to *E. spongiosa*, the black infuscation on forefemora is not limited to the proximal half, often extended to the full length of the anterior surface of the femur (Figs. 3.9, 3.10).

Females: Body length 1.0–4.0mm. Colour: Black except for the following yellow – basal half of scape, posterior half profemora, mesofemora, basal pro- and mesotibiae, apex of hindlegs, tip of ovipositor sheath, tarsomeres 1–4, wing veins (Figs. 3.9, 3.10).

Head: Head 1.3X as broad as high, umbilicate punctured with small tentorial pits. Genal carina present; malar space 0.8X eye height, supraclypeal area smooth. Toruli positioned slightly above lower ocular line. Funicular segments subequal in size; pedicel chalice-shaped; funicle fusiform; F1 slightly narrowed basally, funicular segments with 2 rows of longitudinal sensilla and 3 whorls of setae; clava bisegmented. Ratio of LOL: OOL: POL is 1: 1.2: 2.5. Head posteriorly with postgenal lamina and postgenal grooves ridged, delimited ventrally by postgenal depression. Postgena sparsely setose.

Mesosoma: Umbilicate, 1.5X as long as broad; notauli complete, shallow. Epicnemium imbricate, flattened, with superficial submedial, shallow depressions to receive forecoxae. Mesepisternum anterior to femoral depression umbilicate; mesepimeron reticulate ventrally,

dorsally delimited from femoral depression by fine carina with femoral depression reticulate. Lateral panels of propodeum and callus umbilicately punctate, distinctly delimited from median area by carinae forming irregular setose cells, median furrow delimited, forming 2 rows of irregular asetose cells. Procoxa imbricate except anterior surface smooth, superficially depress anteriorly for reception of lower head. Mesocoxa lacking lamella. Metacoxa densely setose along anterior margins, glabrate with one row of setae along posterior distal margin. Forewing hyaline, marginal vein and postmarginal vein subequal in length.

Metasoma: Gaster 1.2X as long as mesosoma in lateral view; smooth, anterior edge of gastral tergites microreticulate. Petiole 0.5X as long as broad in dorsal view, with projecting lateral teeth as well as mediodorsal prong. Gaster not laterally compressed, oval shaped and convex in lateral view, ovipositor parallel to horizontal axis. $Gt_{1–3}$ glabrate, Gt_4 with 1–4 setae lateromedially, $Gt_{5–8}$ and apex of ovipositor sheath densely setose.

Male: Body length: 1.7–2.2mm. Colour: Black, yellow areas as described for female. Sculpture as described for female. Antennae with funicular segments pedicellate, F2–F5 each with 2 rows of erect setae and 1 row of longitudinal sensilla (Fig. 3.41); scape with ventral plaque in apical half. Gastral petiole in lateral view cylindrical, in dorsal view length about 1.5X as long as greatest width, 0.6X times length of metacoxa; irregularly reticulate dorsally and ventrally (Fig. 3.38), obliterated laterally.

Remarks: Few consistent morphological differences were found between the two *E. spongiosa* species despite deep divergence in COI sequences and differences in host records (See Chapter 2). Considering there are examples of other members of the *Eurytoma rosae* species group having been identified as genetically distinct but morphologically indistinguishable (Ács *et al.*

2002, Gómez *et al.* 2011), *E. spongiosa*2 is the tentative manuscript name assigned for this unique haplogroup. Official description will be provided at time of publication.

Biology: Reared from field populations of galls induced by *Diplolepis ignota, D. nebulosa, D. polita,* and *D. variabilis* on *Rosa* spp.

Material Examined (28♀, 25♂): ALBERTA: Coaldale, 12.V.2007, J.D. & M.R. Shorthouse, ex. *Diplolepis ignota* on *Rosa arkansana* (4♀, 2♂, JDSC). **ONTARIO**: Cochrane, 24.IV.2010, J.D. Shorthouse & Y.M. Zhang, ex. *Diplolepis triforma* on *Rosa acicularis*, (11♀, 7♂, JDSC); Manitoulin Island, 2.V.2009, J.D. Shorthouse & J.D. Renelli, ex. *Diplolepis triforma* on *Rosa acicularis*, (1♀, 3♂, JDSC); Manitoulin Island, 6.V.2010, J.D. Shorthouse & Y.M. Zhang, ex. *Diplolepis triforma* on *Rosa canina*, (1♀, 5♂, JDSC); Manitoulin Island, 4.IX.2010, J.D. & M.R. Shorthouse, ex. *Diplolepis nebulosa* on *Rosa blanda*, (1♀, JDSC); Moose Factory Island, 13.VIII.1998, J.D. Shorthouse & M.G. St. John, ex. *Diplolepis polita* on *Rosa acicularis* (1♂, JDSC); Renfrew, 15.IV.2000, J.D. Shorthouse, ex. *Diplolepis fusiformans* on *Rosa blanda* (1♂, JDSC). **QUÉBEC**: La Sarre, 13.VII.2010, B.L. Smallwood & Y.M. Zhang, ex. *Diplolepis polita* on *Rosa acicularis* (5♀, 1♂, JDSC). **SASKATCHEWAN**: Maple Creek, 10.V.2003, J.D. & M.R. Shorthouse, ex. *Diplolepis ignota* on *Rosa arkansana* (5♀, 5♂, JDSC).

Distribution: From Alberta, Ontario, Québec, and Saskatchewan (Fig. 3.47).

***Tenuipetiolus ruber* Bugbee**

(Figs. 3.13, 3.14, 3.16, 3.21, 3.25, 3.28, 3.31)

Tenuipetiolus rubra (Bugbee) 1951a: 39–42. Holotype ♀ (USNM). Type data: USA, Indiana [Bloomington]; reared from galls induced by *Diplolepis rosae* (L.) on species of *Rosa*, March 28, 1939.

Females: Body length 2.0–3.6 mm. Colour: Black except for the following yellow to brown: Basal half of scape, foretibiae, apices of all femora and meso- hind tibiae, tip of ovipositor sheath, tegulae, wing veins. Tarsomeres 1-4 white (Figs. 3.13).

Head: Head 1.4X as broad as high, umbilicate punctured with small tentorial pits. Genal carina absent; malar space 0.8X eye height, clypeus emarginate and supraclypeal area superficially rugose. Toruli positioned about submedial to lower ocular line. Antennae with funicular segments subequal in size; pedicel chalice-shaped; funicle fusiform; F1 slightly narrowed basally, funicular segments with 1 row of longitudinal sensilla and 2 whorls of setae. Ratio of LOL: OOL: POL is 1: 1: 2.5. Head posteriorly without postgenal lamina or postgenal depression. Postgena sparsely setose and postgenal depression absent (Fig. 3.21).

Mesosoma: Umbilicate, 1.2X as long as broad; notauli incomplete, shallow. Epicnemium imbricate, flattened, with circular median areola present dorsally (Fig. 3.25); Epicnemium superficial submedial, shallow depressions to receive forecoxae. Mesepisternum anterior to femoral depression umbilicate; mesepimeron reticulate ventrally, dorsally delimited from femoral depression by fine carina with femoral depression. Propodeum broadly flattened, superficially punctate, bordered mediolaterally by numerous carinae forming irregular setose cells, median furrow not delimited (Fig. 3.28). Procoxa imbricate except anterior surface smooth, superficially depress anteriorly for reception of lower head. Mesocoxa lacking lamella. Metacoxa

asetose anteriorly and one row of setae on the posterior apical margin. Forewing hyaline, marginal vein distinctly longer than postmarginal vein in length.

Metasoma: Gaster 1.5X as long as mesosoma in lateral view; Smooth, anterior edge of gastral tergites microreticulate (Fig. 3.31). Petiole 4.3X as long as broad in dorsal view, without projecting lateral teeth, strigose laterally. Gaster laterally compressed, triangular shaped and strongly convex in lateral view, ovipositor parallel to horizontal axis. Gt_1 and Gt_2 fused dorsally, $Gt_{1–4}$ glabrate, $Gt_{5–8}$ and apex of ovipositor sheath setose.

Male: Body length: 1.4–2.5mm. Colour: Black, yellow areas as described for female (Fig. 3.14). Sculpture as described for female. Antennae with funicular segments pedicellate, F2–F5 each with 2 rows of erect setae and 1 row of longitudinal sensilla; scape without ventral plaque. Gastral petiole in lateral view cylindrical, in dorsal view length about 3X as long as greatest width, 2X times length of metacoxa; strigose laterally.

Remarks: This species is likely a generalist that is not restricted to rose cynipid galls. Rearing records of the original description includes cynipids of the genus *Diastrophus*.

Biology: Reared from field populations of galls induced by *Diplolepis bassetti, D. polita, D. nodulosa,* and *D. triforma* on *Rosa* spp.

Material Examined (27♀, 12♂): BRITISH COLUMBIA: Osoyoos, 14.V.2003, J.D. & M.R. Shorthouse, ex. *Diplolepis bassetti* on *Rosa woodsii* (3♀, 3♂, JDSC). **ONTARIO**: Chelmsford, 5.V.1994, S.E. Brooks, ex. *Periclistus pirata*-modified galls of *Diplolepis nodulosa* (3♀, 1♂, JDSC); Cochrane, 24.IV.2010, J.D. Shorthouse & Y.M. Zhang, ex. *Diplolepis triforma* on *Rosa acicularis* (9♀, 2♂, JDSC); Manitoulin Island, 2.V.2009, J.D. Shorthouse & J.D. Renelli, ex.

Diplolepis triforma on *Rosa acicularis* (5♀, 1♂, JDSC). **QUÉBEC**: La Sarre, 13.VII.2010, B.L. Smallwood & Y.M. Zhang, ex. *Diplolepis polita* on *Rosa acicularis* (7♀, 5♂, JDSC).

Distribution: Disjunct populations have been found in Western Canada in British Columbia and Eastern Canada in Ontario and Québec (Fig. 3.49).

Discussion

Taxonomic recognition of eurytomids has proven to be problematic in past studies, as with other members within the genus *Eurytoma*, the species associated with galls of *Diplolepis* are conservative in regards to adult morphology. The distinguishing features presented by Bugbee (1951a, 1951b, 1967, 1973) are often ambiguous due to overlapping measurements and intermediate character states. While the results of this study indicates that morphological characters on the posterior head capsule, male antennae, and petiole were particular useful in species delimitation, they are often obscured on intact specimens and cannot be seen clearly unless dissections are performed. In addition, the presence of the precoxal tooth, which is formed by the raised adscrobal carina on the mesopleuron and the defining characteristic used to distinguish the *rosae* species group (Lotfalizadeh *et al.* 2007a), is often inconspicuous and sometimes absent in smaller specimens. The overall morphological similarities between the Canadian species of *Eurytoma* with other members of the *rosae* species group found in Europe suggest that these species shared a common evolutionary line. As the *rosae* group is most diverse in Europe (Lotfalizadeh *et al.* 2007b, G. Delvare pers. comm.), it seems likely that the six *Eurytoma* species found in Canada represent an extension of this species group, although the low

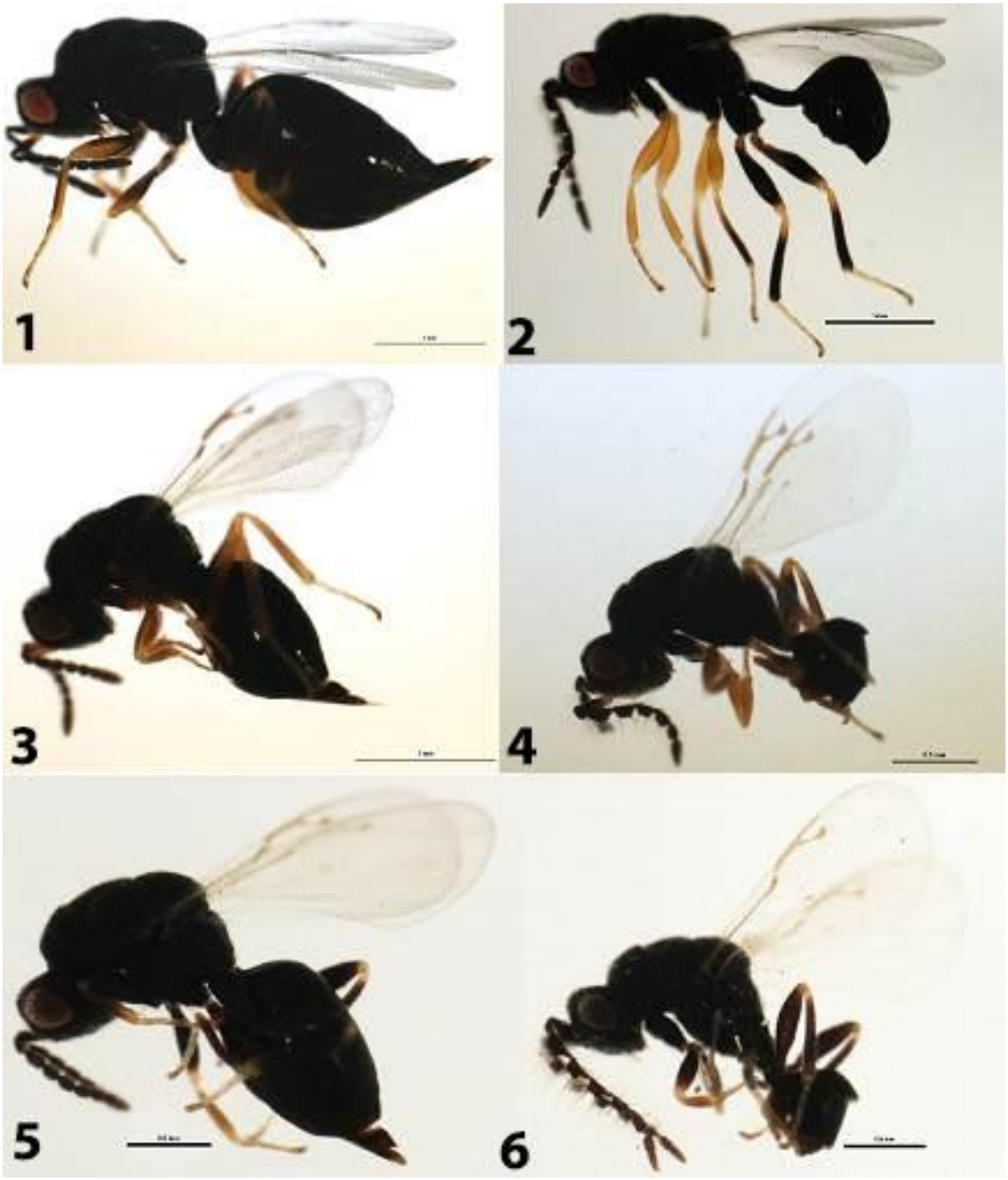
number of Nearctic *rosae* group species could simply be the result of insufficient taxonomic study.

While the ranges of this study (Figs. 3.43 – 3.49) only include localities within Canada, it is likely representative of the Nearctic fauna as the diversity of their host plant, wild roses are predominantly found within Canada (Shorthouse 2010). The distribution of the seven eurytomid species has been greatly expanded, and while the full ranges of these eurytomids are unknown, the distribution likely mirrors that of their hosts. The eurytomids in this study exhibit a trascontinental distribution within Canada, extending to the northern extreme of wild roses near the Hudson Bay Lowlands. The only exceptions being *E. obtusilobae* (Fig. 3.46) and *T. ruber* (Fig. 3.49), as they are only found in disjunct populations in Canada, although past literature suggests it is widespread within USA (Bugbee 1951a, 1951b). As a result of this study, new provincial records were recorded from British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Québec, New Brunswick, and Prince Edward Island since the last revision (Bugbee 1951a, 1951b). Bugbee used distributions as an important criterion for delimiting species of *Eurytoma* (1951b, 1967, 1973), the range expansion in this study joins the previously disjunct populations and along with molecular data (see Chapter 2) supports the synonymizations of these species (e.g. *E. acuta*, *E. calcarea* under *E. discordans*).

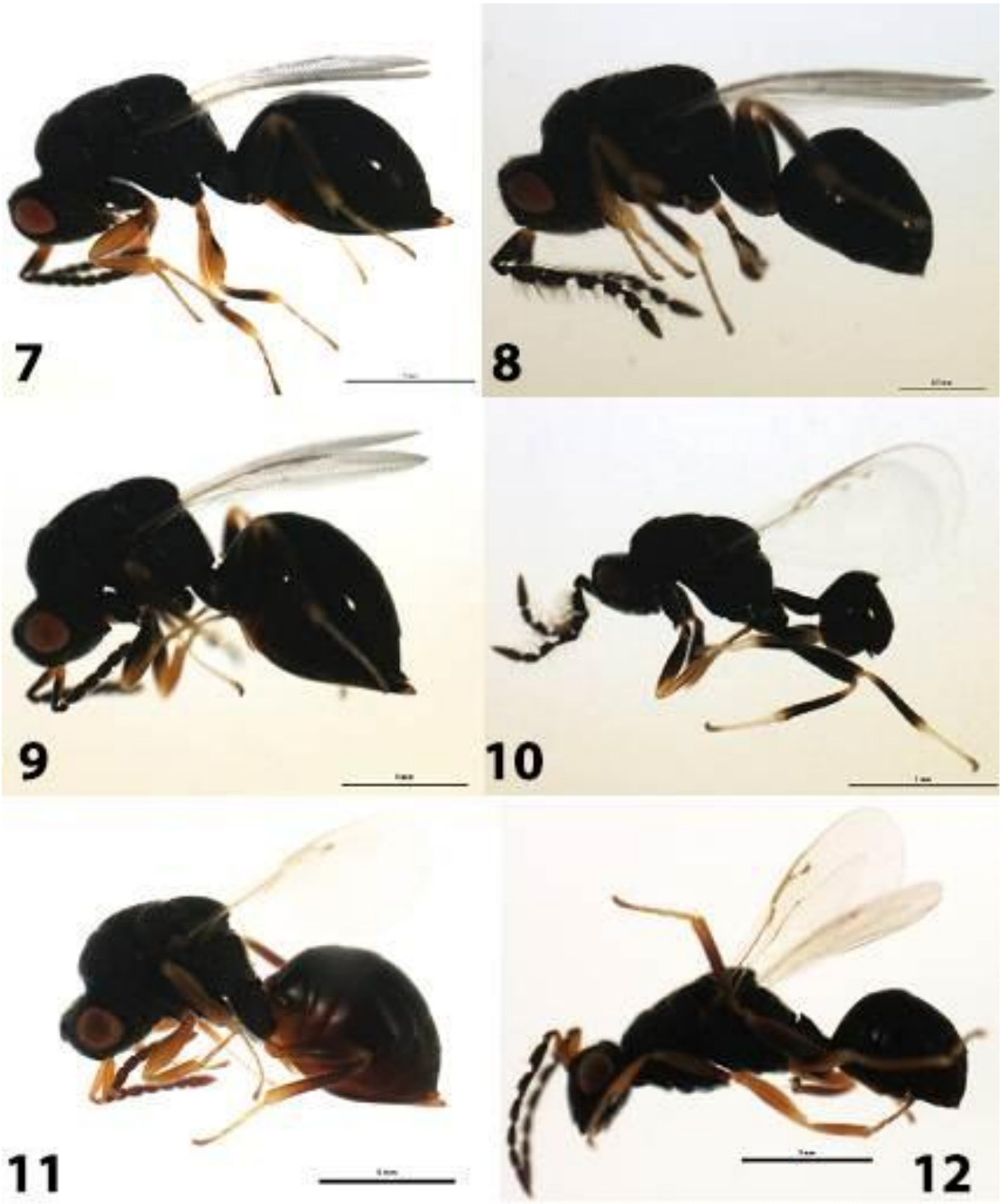
In addition to the galls of *Diplolepis*, *E. discordans* and *T. ruber* have also been collected from galls of *Diastrophus* spp. on raspberry. Given the polyphagous nature of many of these eurytomid species, the full host range is likely much wider than currently known. Thus, using host records alone to distinguish morphologically similar species is error-prone and a source of confusion when identifying eurytomids (Lotfalizadeh *et al.* 2007b). While most eurytomids are

primary parasitoids of the gall inducer, *E. iniquus* has been speculated as a parasitoid of the gall inquiline *Periclistus* rather than the gall inducer. While inquiline-specific parasitoids in association with oak cynipid galls have been reported in the past (Csóka *et al.* 2005), this is the first reported case for the cynipid galls on roses.

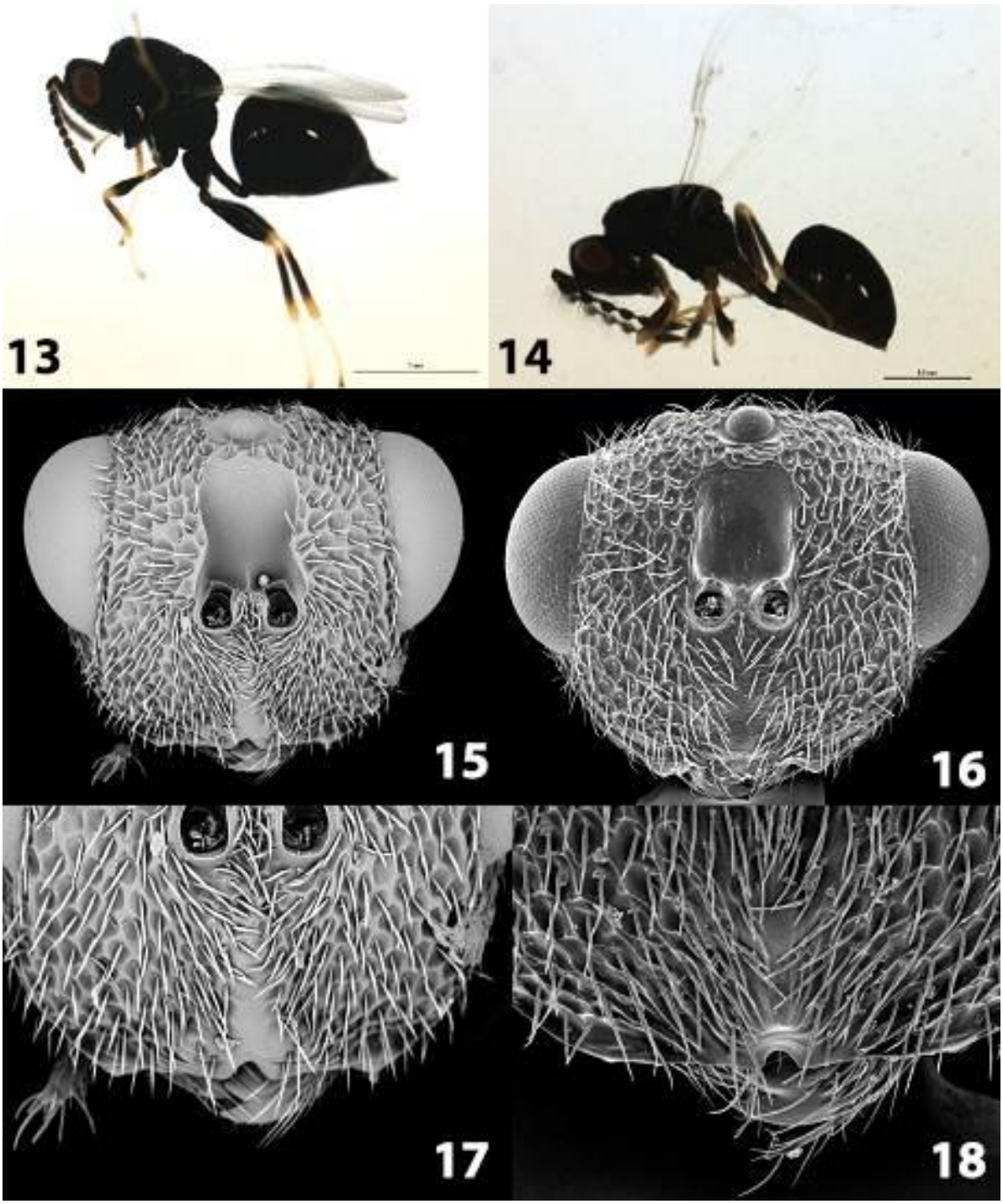
The systematic placement of the Eurytomidae within the superfamily Chalcidoidea has been controversial in past studies (Lotfalizadeh *et al.* 2007b, Gates 2008). The species treated by Bugbee (e.g. 1951a, 1951b, 1967) undoubtedly includes many synonymous species, and a revision of all Nearctic eurytomids is needed. With further insight into the taxonomy of eurytomids associated with cynipid rose galls, a new and exciting approach has been provided for future phylogenetic studies of the whole superfamily Chalcidoidea.



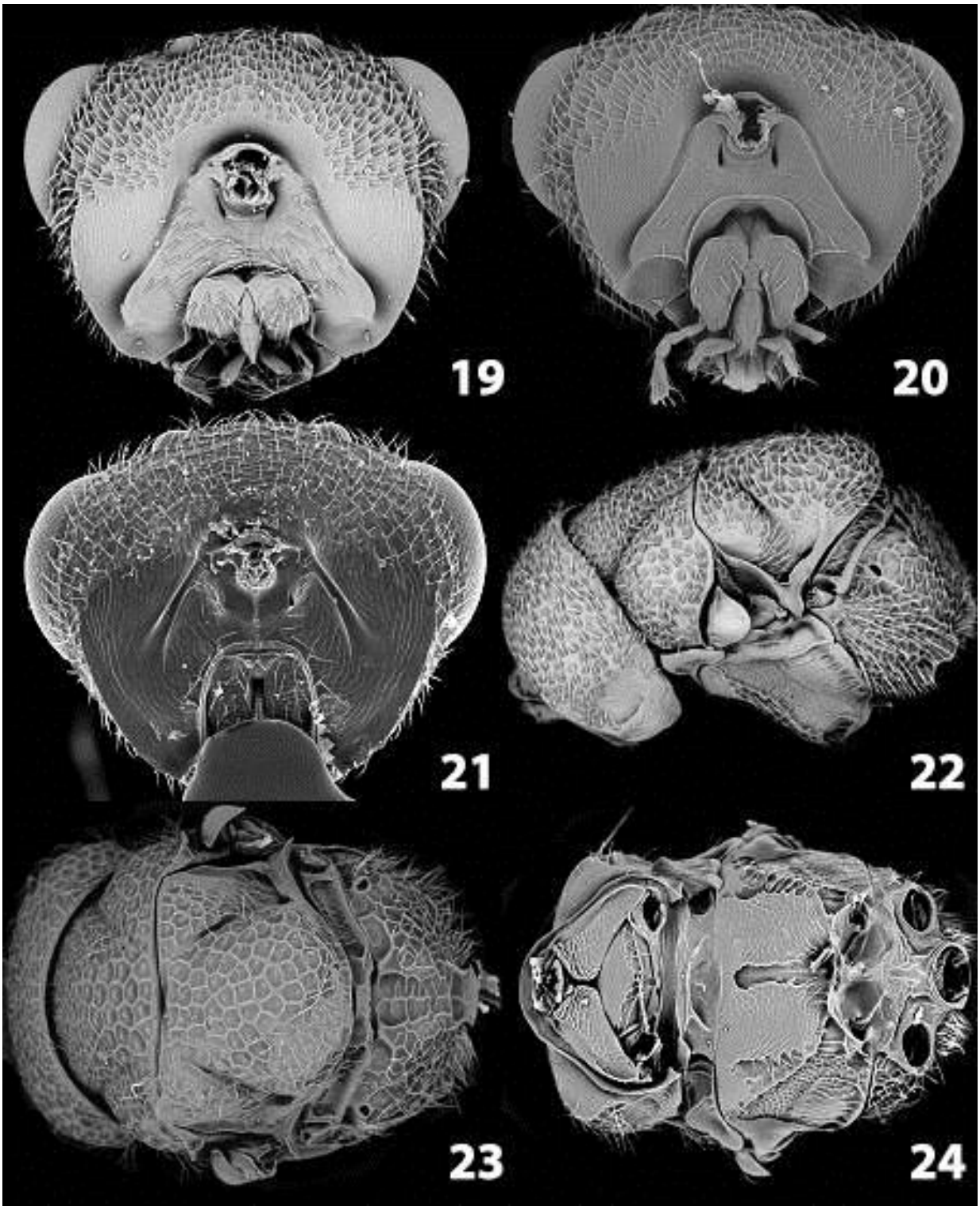
Figures 3.1–3.6. 3.1, *Eurytoma discordans* female habitus; 3.2, *E. discordans* male habitus; 3.3, *Eurytoma iniquus* female habitus; 3.4, *E. iniquus* male habitus; 3.5, *Eurytoma longavena* female habitus; 3.6, *E. longavena* male habitus.



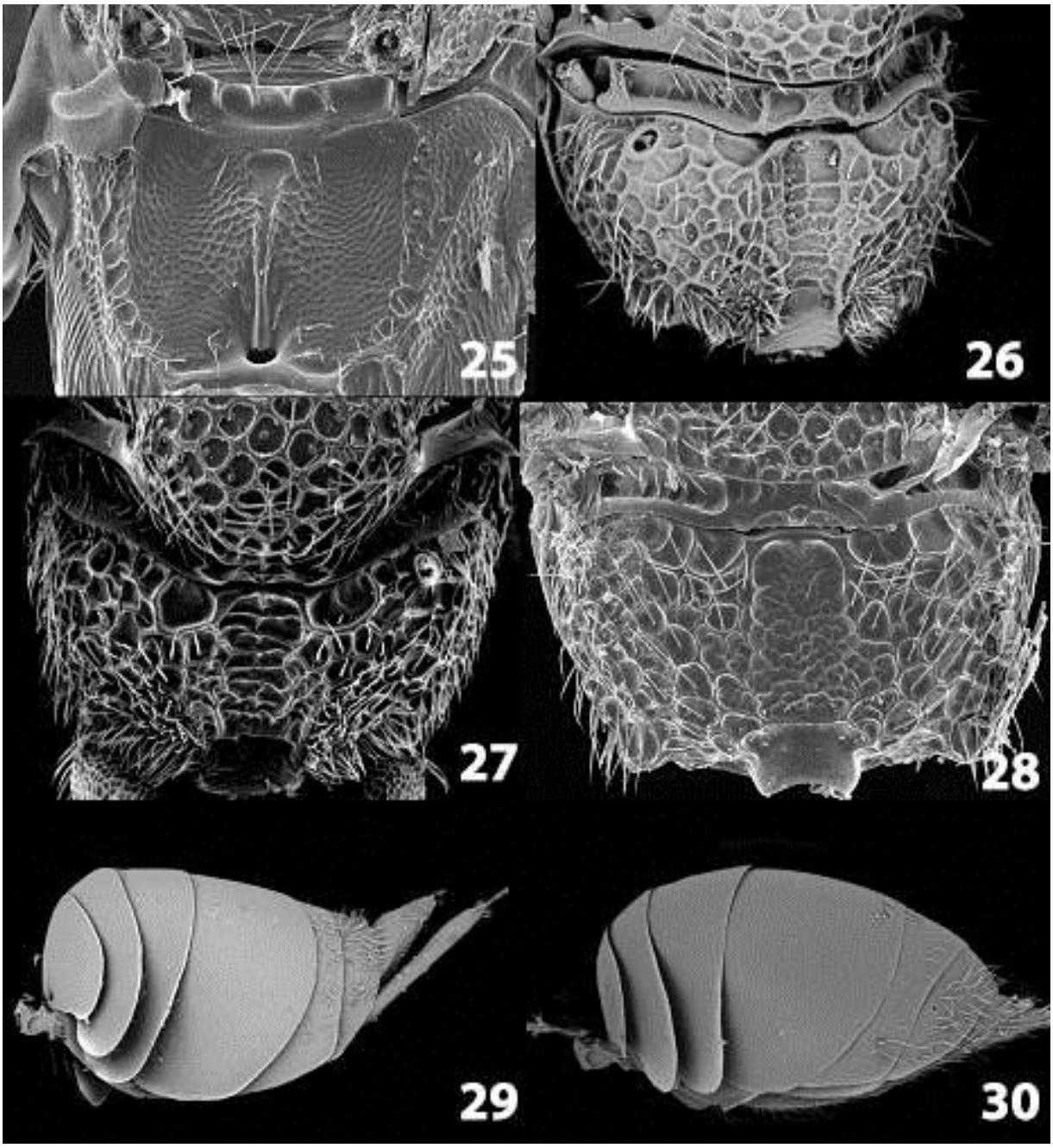
Figures 3.7–3.12. 3.7, *Eurytoma spongiosa* female habitus; 3.8, *E. spongiosa* male habitus; 3.9, *Eurytoma spongiosa*2 female habitus; 3.10, *E. spongiosa*2 male habitus; 3.11, *Eurytoma obtusilobae* female habitus; 3.12, *E. obtusilobae* male habitus.



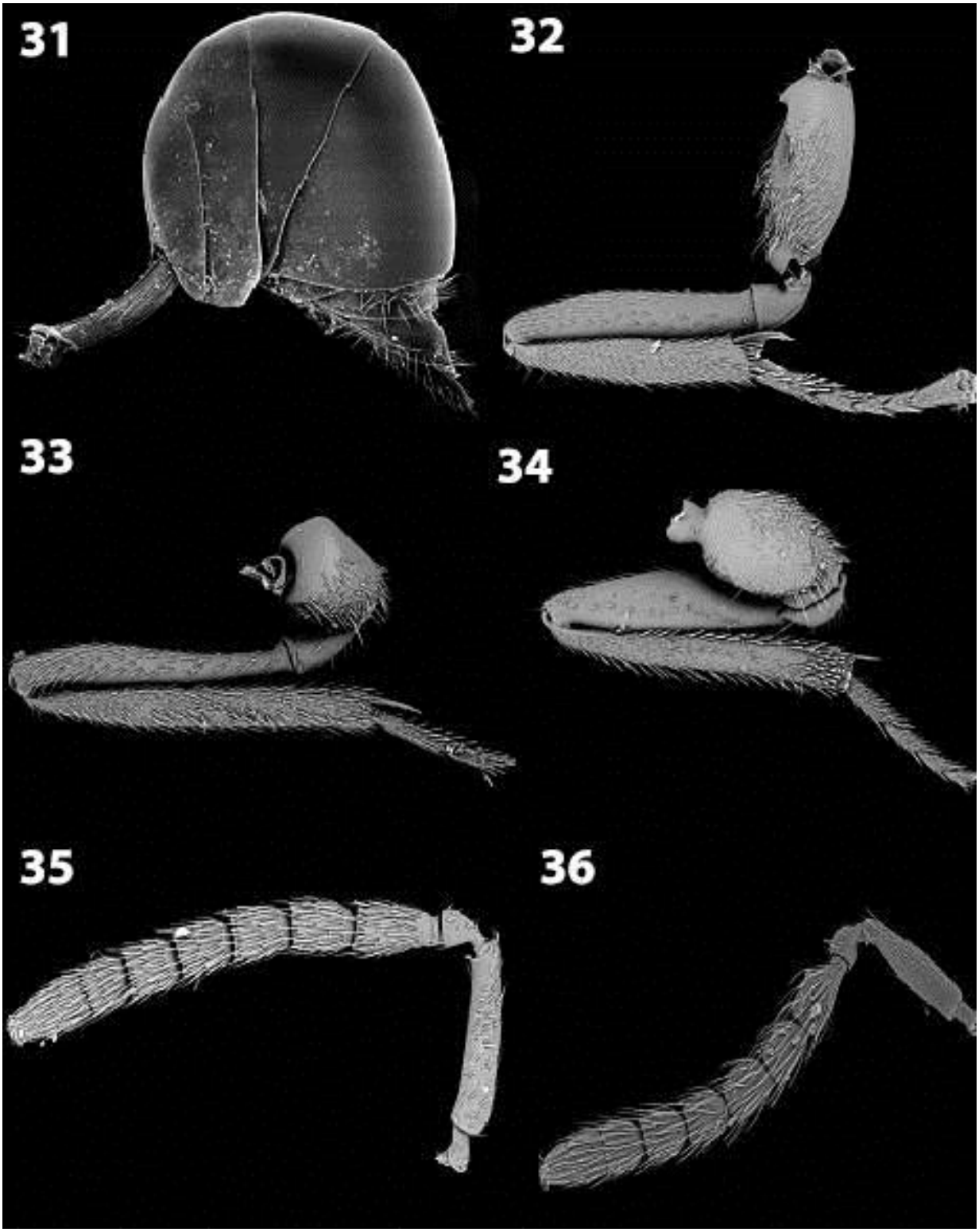
Figures 3.13–3.18. . 3.13, *Tenuipetiolus ruber* female habitus; 3.14, *Tenuipetiolus ruber* male habitus; 3.15, *E. discordans* head anterior; 3.16, *T. ruber* head anterior; 3.17, *E. discordans* clypeus; 3.18, *E. obtusilobae* clypeus.



Figures 3.19–3.24. 3.19, *E. discordans* head posterior; 3.20, *E. longavena* head posterior; 3.21, *T. ruber* head posterior; 3.22, *E. discordans* mesosoma lateral; 3.23, *E. longavena* mesosoma dorsal; 3.24, *E. longavena* mesosoma ventral.



Figures. 3.25–3.30. 3.25, *T. ruber* mesosoma ventral; 3.26, *E. discordans* propodeum; 3.27, *E. obtusilobae* propodeum; 3.28, *T. ruber* propodeum; 3.29, *E. discordans* female metasoma; 3.30, *E. longavena* female metasoma.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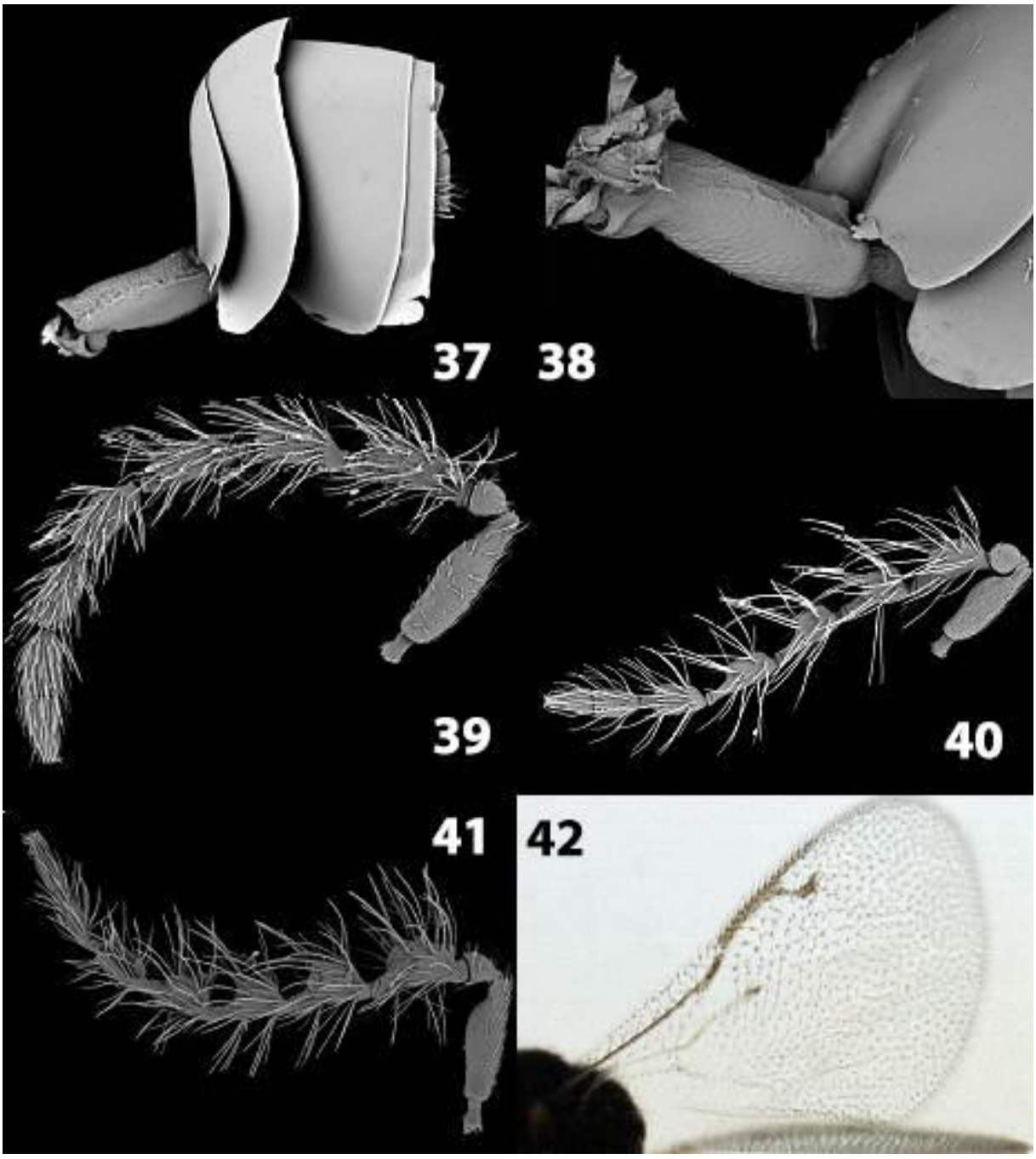
Figures 3.31–3.36. 3.31, *T. ruber* female metasoma; 3.32, *E. discordans* foreleg; 3.33, *E. discordans* midleg, 3.34, *E. discordans* hindleg; 3.35, *E. discordans* female antenna; 3.36, *E. longavena* female antenna.



Figures 3.37–3.42. 3.37, *E. discordans* male metasoma; 3.38, *E. spongiosa*2 male petiole; 3.39, *E. discordans* male antenna, 3.40, *E. longavena* male antenna; 3.41, *E. spongiosa*2 male antenna; 3.42, *E. longavena* forewing.

Figure 3.43. Known localities of *E. discordans* in Canada.

Figure 3.44. Known localities of *E. iniquus* in Canada.

Figure 3.45. Known localities of *E. longavena* in Canada.

Figure 3.46. Known localities of *E. obtusilobae* in Canada.

Figure 3.47. Known localities of *E. spongiosa* in Canada.

Figure 3.48. Known localities of *E. spongiosa*2 in Canada.

Figure 3.49. Known localities of *T. ruber* in Canada.

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Chapter 4 - Conclusion & Future Work

This study examined the diversity of eurytomids associated with galls induced by cynipid wasps of the genus *Diplolepis* in Canada, and provided the first phylogenetic study of the Nearctic eurytomids. An integrative taxonomy approach comprised of morphological characters, mitochondrial DNA, ecological, and geographical data was used to delimit species. The utility of COI for species identification in eurytomids was confirmed by this study, which not only separated morphologically distinct species, but also detected high levels of intraspecific divergence in species complexes. By building a COI reference library for these species, all life stages and sexes can be reliably identified not only by specialists but by those unfamiliar with eurytomid taxonomy.

The resulting phylogeny was used as support for confirmation of 7 eurytomid species, including the synonymization of 4 species of *Eurytoma,* and 1 new manuscript species. Additional host and distribution records were established, many of which are new Canadian records. The monophyly of the subfamily Eurytominae and the *Eurytoma rosae* group is supported by the phylogenetic analysis. A morphologically similar, but genetically and ecologically distinct species tentatively called *E. spongiosa* 2 is discovered as a result of this study in addition to *E. spongiosa*. It was also determined that *E. acuta* and *E. calcarea* are junior synonyms of *E. discordans*; while *E. hebes* and *E. imminuta* are junior synonyms of *E. longavena* and *E. spongiosa*, respectively. New morphological characters were identified for male eurytomids, which were previously unidentifiable and a dichotomous key for both sexes were provided for all 7 species.

Among the 7 eurytomid species, there was strong evidence supporting the bivoltine nature of *E. longavena* and *E. spongiosa* 1, which parasitize inducers of both spring and fall initiated galls. *Eurytoma iniquus* is likely a parasitoid of *Periclistus* inquilines; however, more specimens with accurate host records are needed to further assess this relationship. In addition, *E. discordans* and *T. ruber* are not restricted to cynipid rose galls as they have also been recorded in association with cynipid galls on raspberry.

Although is the data presented here are likely representative of the Nearctic fauna as they cover a broad sampling range, final determination of the natural range of these species will require specimens from the entire host range. Moreover, the addition of other molecular markers, along with morphological studies on the larval eurytomids for future studies will strengthen species boundaries and aid in the clarification of species complexes such as *E. discordans*. An integrative approach could also be used to delimit other species of Nearctic eurytomids, particularly members of the large genus *Eurytoma*. Only a small percentage of Nearctic *Eurytoma* are associated with galls of *Diplolepis*, while most species are likely associated with numerous hosts and the same techniques used in this thesis could be applied to the entire genus.

The results of this study highlight the taxonomic problems associated with Nearctic hymenopteran parasitoids as revisions are needed for nearly all taxa. The diverse fauna and the taxonomic challenges of Eurytomidae as a representative taxa for microhymenoptera, provides unlimited opportunity to unite independent datasets, and a model taxa for future taxonomic work.

Appendix I. Locality for all specimens

Sample ID	Species	Sex	Extra Info	Collectors	Collection Date	Province	Locality	Exact Site	Lat	Lon
MZEUR-0001	Eurytoma spongiosa1	F	Ex. D. triforma on R. canina	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0002	Eurytoma spongiosa1	F	Ex. D. triforma on R. canina	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0004	Eurytoma spongiosa2	F	Ex. D. triforma on R. canina	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0005	Eurytoma spongiosa1	F	Ex. D. triforma on R. canina	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0006	Eurytoma spongiosa1	F	Ex. D. triforma on R. canina	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0007	Eurytoma spongiosa1	F	Ex. D. triforma on R. canina	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0009	Eurytoma spongiosa1	F	Ex. D. triforma on R. canina	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0010	Eurytoma spongiosa1	F	Ex. D. triforma on R. canina	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0011	Eurytoma spongiosa1	M	Ex. D. triforma on R. canina	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0014	Eurytoma spongiosa1	M	Ex. D. triforma on R. canina	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0020	Eurytoma spongiosa1	M	Ex. D. triforma on R. canina	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0022	Eurytoma spongiosa1	F	Ex. D. spinosa on R. rugosa	J. D. Shorthouse & M. R. Shorthouse	19-Apr-2006	Ontario	Kanata	Parking lot of IKEA Shopping Mall on Highway 417 West	45.350	-75.785
MZEUR-0031	Eurytoma spongiosa1	M	Ex. D. spinosa on R. rugosa	J. D. Shorthouse & M. R. Shorthouse	19-Apr-2006	Ontario	Kanata	Parking lot of IKEA Shopping Mall on Highway 417 West	45.350	-75.785
MZEUR-0032	Eurytoma spongiosa1	M	Ex. D. spinosa on R. rugosa	J. D. Shorthouse & M. R. Shorthouse	19-Apr-2006	Ontario	Kanata	Parking lot of IKEA Shopping Mall on Highway 417 West	45.350	-75.785
MZEUR-0035	Eurytoma spongiosa1	M	Ex. D. spinosa on R. rugosa	J. D. Shorthouse & M. R. Shorthouse	19-Apr-2006	Ontario	Kanata	Parking lot of IKEA Shopping Mall on Highway 417 West	45.350	-75.785
MZEUR-0037	Eurytoma spongiosa1	M	Ex. D. spinosa on R. rugosa	J. D. Shorthouse & M. R. Shorthouse	19-Apr-2006	Ontario	Kanata	Parking lot of IKEA Shopping Mall on Highway 417 West	45.350	-75.785
MZEUR-0039	Eurytoma spongiosa1	M	Ex. D. spinosa on R. rugosa	J. D. Shorthouse & M. R. Shorthouse	19-Apr-2006	Ontario	Kanata	Parking lot of IKEA Shopping Mall on Highway 417 West	45.350	-75.785
MZEUR-0045	Eurytoma spongiosa1	F	Ex. D. spinosa on R. rugosa	J. D. Shorthouse & M. R. Shorthouse	19-Apr-2006	Ontario	Kanata	Parking lot of IKEA Shopping Mall on Highway 417 West	45.310	-75.921
MZEUR	Eurytoma	M	Ex. D. spinosa	J. D. Shorthouse & M. R.	19-Apr-	Ontario	Kanata	Parking lot of IKEA	45.310	-75.921

-0051	spongiosa1		on R. rugosa	Shorthouse	2006			Shopping Mall on Highway 417 West		
MZEUR-0067	Eurytoma spongiosa2	F	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0068	Eurytoma spongiosa2	F	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0069	Eurytoma spongiosa2	F	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0070	Eurytoma spongiosa2	F	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0071	Eurytoma spongiosa2	F	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0072	Eurytoma spongiosa2	F	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0073	Eurytoma spongiosa2	F	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0074	Eurytoma spongiosa2	F	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0075	Eurytoma spongiosa2	F	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0076	Eurytoma spongiosa2	F	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0077	Eurytoma spongiosa2	M	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0078	Eurytoma spongiosa2	M	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0079	Eurytoma spongiosa2	M	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0080	Eurytoma spongiosa2	M	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0081	Eurytoma spongiosa2	M	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0082	Eurytoma spongiosa2	M	Ex D. triforma on R.	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017

			acicularis							
MZEUR-0084	Eurytoma spongiosa2	M	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0085	Tenuipetiolus ruber	M	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0086	Tenuipetiolus ruber	M	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0094	Tenuipetiolus ruber	F	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0096	Eurytoma spongiosa2	F	Ex D. triforma on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0097	Eurytoma spongiosa1	F	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0098	Eurytoma spongiosa1	F	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0099	Eurytoma spongiosa1	F	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0100	Eurytoma spongiosa1	F	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0101	Eurytoma spongiosa1	F	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0102	Eurytoma spongiosa1	F	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0103	Eurytoma spongiosa2	F	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0104	Eurytoma spongiosa1	F	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0105	Eurytoma spongiosa1	F	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0106	Eurytoma spongiosa1	F	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0107	Eurytoma spongiosa1	M	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0108	Eurytoma spongiosa1	M	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0109	Eurytoma spongiosa2	M	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0110	Eurytoma spongiosa1	M	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0111	Eurytoma spongiosa1	M	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0112	Eurytoma spongiosa2	M	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300

MZEUR-0113	Eurytoma spongiosa2	M	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0114	Eurytoma spongiosa2	M	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0115	Eurytoma spongiosa2	M	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0116	Eurytoma spongiosa1	M	Ex D. triforma on R. canina	J.D. Shorthouse & M.Y. Zhang	06-May-2010	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.831	-82.300
MZEUR-0117	Eurytoma longavena	F	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0118	Eurytoma sp1	F	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0119	Eurytoma longavena	F	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0120	Eurytoma longavena	F	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0121	Eurytoma longavena	F	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0122	Eurytoma longavena	F	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0123	Eurytoma longavena	F	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0124	Eurytoma longavena	F	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0125	Eurytoma longavena	F	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0126	Eurytoma sp1	F	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0127	Eurytoma longavena	M	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0128	Eurytoma longavena	M	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0129	Eurytoma longavena	M	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0130	Eurytoma longavena	M	Ex D. rosaefolii on R.	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017

			acicularis							
MZEUR-0131	Eurytoma longavena	M	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0132	Eurytoma longavena	M	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0133	Eurytoma longavena	M	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0134	Eurytoma iniquus	M	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & S.T. Offman	18-May-2002	Ontario	Red Lake	NW tip of town	51.061	-93.780
MZEUR-0135	Eurytoma iniquus	M	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & S.T. Offman	18-May-2002	Ontario	Red Lake	NW tip of town	51.061	-93.780
MZEUR-0136	Eurytoma sp1	M	Ex D. rosaefolii on R. acicularis	J.D. Shorthouse & S.T. Offman	18-May-2002	Ontario	Red Lake	NW tip of town	51.061	-93.780
MZEUR-0137	Eurytoma spongiosa1	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0138	Eurytoma spongiosa1	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0139	Eurytoma spongiosa1	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0140	Eurytoma spongiosa1	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0141	Eurytoma spongiosa1	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR-0142	Eurytoma spongiosa1	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR-0143	Eurytoma spongiosa1	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR-0144	Eurytoma spongiosa1	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR-0145	Eurytoma spongiosa1	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR-0146	Eurytoma spongiosa1	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR-0147	Eurytoma discordans	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR-0148	Eurytoma discordans	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR-0149	Eurytoma discordans	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR-0150	Eurytoma discordans	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR	Eurytoma	F	Ex D. spinosa	J.D. Shorthouse & M.Y. Zhang	23-Apr-	Ontario	Moose Factory Island	South Bank	51.249	-80.614

-0151	discordans		on R. blanda		2010					
MZEUR -0152	Eurytoma discordans	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR -0153	Eurytoma discordans	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR -0154	Eurytoma discordans	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR -0155	Eurytoma discordans	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR -0156	Eurytoma discordans	F	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR -0157	Eurytoma discordans	M	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR -0158	Eurytoma discordans	M	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR -0159	Eurytoma discordans	M	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR -0160	Eurytoma discordans	M	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR -0161	Eurytoma discordans	M	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017
MZEUR -0162	Eurytoma discordans	M	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR -0163	Eurytoma discordans	M	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR -0164	Eurytoma discordans	M	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR -0165	Eurytoma discordans	M	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR -0166	Eurytoma discordans	M	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR -0167	Eurytoma spongiosa1	M	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR -0168	Eurytoma spongiosa1	M	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR -0169	Eurytoma spongiosa1	M	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR -0170	Eurytoma spongiosa1	M	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR -0171	Eurytoma spongiosa1	M	Ex D. spinosa on R. blanda	J.D. Shorthouse & M.Y. Zhang	23-Apr-2010	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR -0173	Eurytoma discordans	F	Ex D. radicum on R. acicularis	M.J.T. Bodnar	28-May-2005	Ontario	Fort Albany	Riverbank	52.208	-81.685
MZEUR -0174	Eurytoma discordans	F	Ex D. radicum on R. acicularis	M.J.T. Bodnar	28-May-2005	Ontario	Fort Albany	Riverbank	52.208	-81.685
MZEUR	Eurytoma	F	Ex D. radicum	M.J.T. Bodnar	28-May-	Ontario	Fort Albany	Riverbank	52.208	-81.685

-0176	discordans		on R. acicularis		2005					
			Ex D. radicum							
MZEUR	Eurytoma		on R.		28-May-					
-0178	discordans	F	acicularis	M.J.T. Bodnar	2005	Ontario	Fort Albany	Riverbank	52.208	-81.685
			Ex D. radicum							
MZEUR	Eurytoma		on R.		28-May-					
-0187	discordans	M	acicularis	M.J.T. Bodnar	2005	Ontario	Fort Albany	Riverbank	52.208	-81.685
			Ex D. radicum							
MZEUR	Eurytoma		on R.		28-May-					
-0188	discordans	M	acicularis	M.J.T. Bodnar	2005	Ontario	Fort Albany	Riverbank	52.208	-81.685
MZEUR	Eurytoma		Ex D. bicolor		15-May-					
-0192	iniquus	F	on R. blanda	J.D. Shorthouse & S.T. Offman	2002	Ontario	Thunder Bay	Boundary Road	48.416	-89.267
MZEUR	Eurytoma		Ex D. bicolor		15-May-					
-0193	iniquus	F	on R. blanda	J.D. Shorthouse & S.T. Offman	2002	Ontario	Thunder Bay	Boundary Road	48.416	-89.267
MZEUR	Eurytoma		Ex D. bicolor		15-May-					
-0197	iniquus	F	on R. blanda	J.D. Shorthouse & S.T. Offman	2002	Ontario	Thunder Bay	Boundary Road	48.416	-89.267
MZEUR	Eurytoma		Ex D. bicolor		15-May-					
-0201	iniquus	F	on R. blanda	J.D. Shorthouse & S.T. Offman	2002	Ontario	Thunder Bay	Boundary Road	48.416	-89.267
MZEUR	Eurytoma		Ex D. polita on	B.L. Smallwood & M.Y.	13-Jul-					
-0216	longavena	F	R. acicularis	Zhang	2010	Quebec	La Sarre	roadside near agricultural area	48.807	-79.205
MZEUR	Eurytoma		Ex D. polita on	B.L. Smallwood & M.Y.	13-Jul-					
-0217	longavena	F	R. acicularis	Zhang	2010	Quebec	La Sarre	roadside near agricultural area	48.807	-79.205
MZEUR	Eurytoma		Ex D. polita on	B.L. Smallwood & M.Y.	13-Jul-					
-0218	longavena	F	R. acicularis	Zhang	2010	Quebec	La Sarre	roadside near agricultural area	48.807	-79.205
MZEUR	Eurytoma		Ex D. polita on	B.L. Smallwood & M.Y.	13-Jul-					
-0219	longavena	F	R. acicularis	Zhang	2010	Quebec	La Sarre	roadside near agricultural area	48.807	-79.205
MZEUR	Eurytoma		Ex D. polita on	B.L. Smallwood & M.Y.	13-Jul-					
-0220	spongiosa2	F	R. acicularis	Zhang	2010	Quebec	La Sarre	roadside near agricultural area	48.807	-79.205
MZEUR	Eurytoma		Ex D. polita on	B.L. Smallwood & M.Y.	13-Jul-					
-0221	spongiosa2	F	R. acicularis	Zhang	2010	Quebec	La Sarre	roadside near agricultural area	48.807	-79.205
MZEUR	Eurytoma		Ex D. polita on	B.L. Smallwood & M.Y.	13-Jul-					
-0223	spongiosa2	F	R. acicularis	Zhang	2010	Quebec	La Sarre	roadside near agricultural area	48.807	-79.205
MZEUR	Eurytoma		Ex D. polita on	B.L. Smallwood & M.Y.	13-Jul-					
-0226	longavena	F	R. acicularis	Zhang	2010	Quebec	La Sarre	roadside near agricultural area	48.807	-79.205
MZEUR	Eurytoma		Ex D. polita on	B.L. Smallwood & M.Y.	13-Jul-					
-0229	spongiosa2	M	R. acicularis	Zhang	2010	Quebec	La Sarre	roadside near agricultural area	48.807	-79.205
MZEUR	Tenuipetiolu		Ex D. polita on	B.L. Smallwood & M.Y.	13-Jul-					
-0230	s ruber	M	R. acicularis	Zhang	2010	Quebec	La Sarre	roadside near agricultural area	48.807	-79.205
MZEUR	Tenuipetiolu		Ex D. polita on	B.L. Smallwood & M.Y.	13-Jul-					
-0231	s ruber	M	R. acicularis	Zhang	2010	Quebec	La Sarre	roadside near agricultural area	48.807	-79.205
MZEUR	Eurytoma		Ex D. polita on	B.L. Smallwood & M.Y.	13-Jul-					
-0232	longavena	M	R. acicularis	Zhang	2010	Quebec	La Sarre	roadside near agricultural area	48.807	-79.205
			Ex. D.							
MZEUR	Eurytoma		nebulosa on R.		04-Sep-					
-0239	spongiosa2	F	blanda	J.D. and M.R. Shorthouse	2010	Ontario	Manitoulin Island	Providence Bay	45.677	-82.270
			Ex. D.							
MZEUR	Eurytoma		nebulosa on R.		04-Sep-					
-0240	longavena	F	blanda	J.D. and M.R. Shorthouse	2010	Ontario	Manitoulin Island	Providence Bay	45.677	-82.270

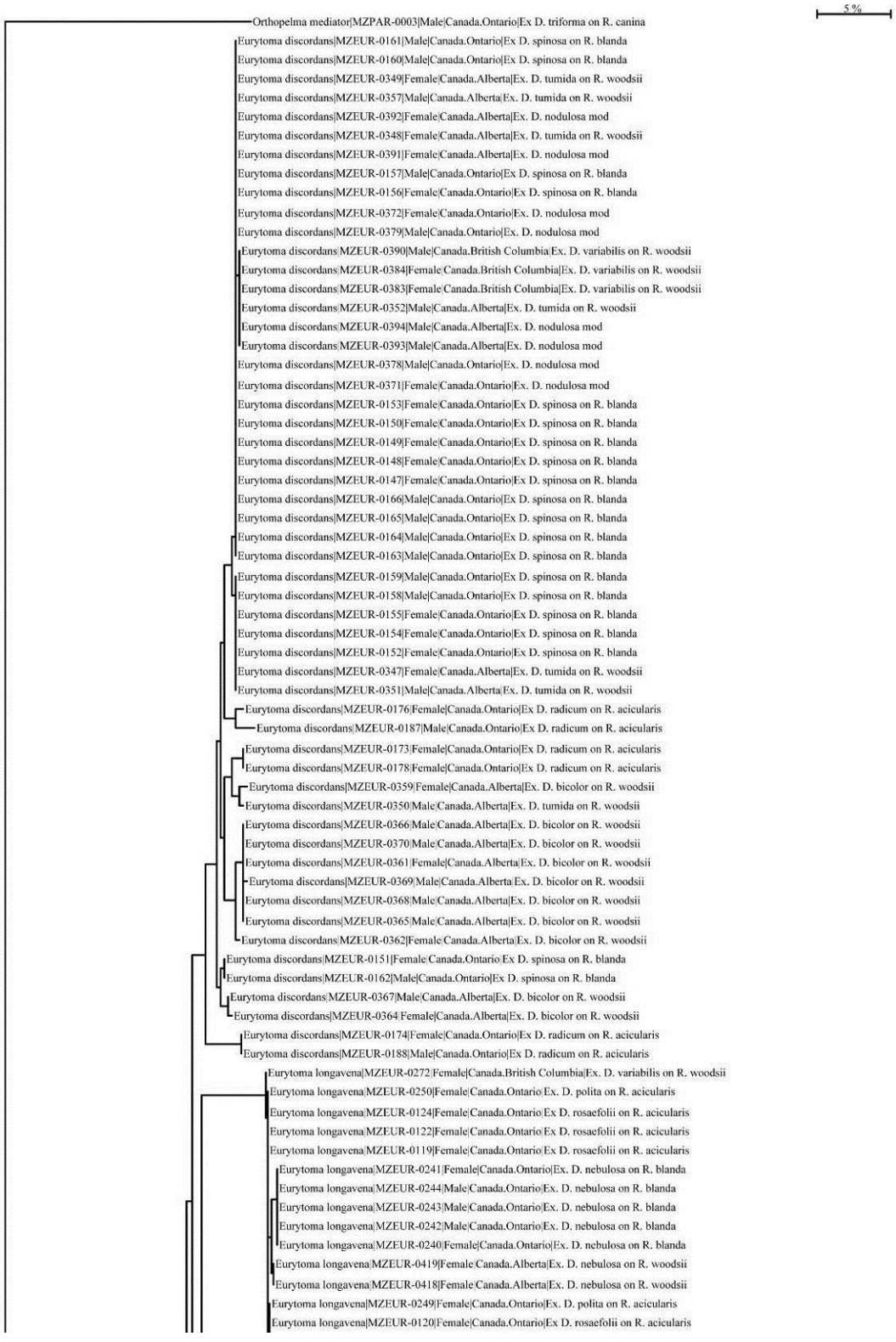
MZEUR-0241	Eurytoma longavena	F	Ex. D. nebulosa on R. blanda	J.D. and M.R. Shorthouse	04-Sep-2010	Ontario	Manitoulin Island	Providence Bay	45.677	-82.270
MZEUR-0242	Eurytoma longavena	M	Ex. D. nebulosa on R. blanda	J.D. and M.R. Shorthouse	04-Sep-2010	Ontario	Manitoulin Island	Providence Bay	45.677	-82.270
MZEUR-0243	Eurytoma longavena	M	Ex. D. nebulosa on R. blanda	J.D. and M.R. Shorthouse	04-Sep-2010	Ontario	Manitoulin Island	Providence Bay	45.677	-82.270
MZEUR-0244	Eurytoma longavena	M	Ex. D. nebulosa on R. blanda	J.D. and M.R. Shorthouse	04-Sep-2010	Ontario	Manitoulin Island	Providence Bay	45.677	-82.270
MZEUR-0245	Eurytoma longavena	M	Ex. D. nebulosa on R. blanda	J.D. and M.R. Shorthouse	04-Sep-2010	Ontario	Manitoulin Island	Providence Bay	45.677	-82.270
MZEUR-0247	Eurytoma longavena	F	Ex. D. polita on R. acicularis	J.D. Shorthouse and M. G. St. John	13-Aug-1998	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR-0248	Eurytoma longavena	F	Ex. D. polita on R. acicularis	J.D. Shorthouse and M. G. St. John	13-Aug-1998	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR-0249	Eurytoma longavena	F	Ex. D. polita on R. acicularis	J.D. Shorthouse and M. G. St. John	13-Aug-1998	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR-0250	Eurytoma longavena	F	Ex. D. polita on R. acicularis	J.D. Shorthouse and M. G. St. John	13-Aug-1998	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR-0253	Eurytoma spongiosa2	M	Ex. D. polita on R. acicularis	J.D. Shorthouse and M. G. St. John	13-Aug-1998	Ontario	Moose Factory Island	South Bank	51.249	-80.614
MZEUR-0272	Eurytoma longavena	F	Ex. D. variabilis on R. woodsii	J.D. Shorthouse and R. G. Lalonde	15-Oct-1999	British Columbia	Kelowna	2km S. E. of Kelowna airport	49.952	-119.381
MZEUR-0310	Tenuipetiolus ruber	F	Ex. D. triforma on R. acicularis	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0312	Tenuipetiolus ruber	F	Ex. D. triforma on R. acicularis	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0314	Tenuipetiolus ruber	F	Ex. D. triforma on R. acicularis	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0315	Eurytoma spongiosa2	M	Ex. D. triforma on R. acicularis	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0316	Eurytoma spongiosa2	M	Ex. D. triforma on R. acicularis	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337

MZEUR-0319	Eurytoma spongiosa2	M	Ex. D. triforma on R. acicularis	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZEUR-0347	Eurytoma discordans	F	Ex. D. tumida on R. woodsii	J. D. & M.R. Shorthouse	10-May-2011	Alberta	Township Road 92, Willow Creek No. 26	Head-Smashed-In Buffalo Jump	49.749	-113.633
MZEUR-0348	Eurytoma discordans	F	Ex. D. tumida on R. woodsii	J. D. & M.R. Shorthouse	10-May-2011	Alberta	Township Road 92, Willow Creek No. 26	Head-Smashed-In Buffalo Jump	49.749	-113.633
MZEUR-0349	Eurytoma discordans	F	Ex. D. tumida on R. woodsii	J. D. & M.R. Shorthouse	10-May-2011	Alberta	Township Road 92, Willow Creek No. 26	Head-Smashed-In Buffalo Jump	49.749	-113.633
MZEUR-0350	Eurytoma discordans	M	Ex. D. tumida on R. woodsii	J. D. & M.R. Shorthouse	10-May-2011	Alberta	Township Road 92, Willow Creek No. 26	Head-Smashed-In Buffalo Jump	49.749	-113.633
MZEUR-0351	Eurytoma discordans	M	Ex. D. tumida on R. woodsii	J. D. & M.R. Shorthouse	10-May-2011	Alberta	Township Road 92, Willow Creek No. 26	Head-Smashed-In Buffalo Jump	49.749	-113.633
MZEUR-0352	Eurytoma discordans	M	Ex. D. tumida on R. woodsii	J. D. & M.R. Shorthouse	10-May-2011	Alberta	Township Road 92, Willow Creek No. 26	Head-Smashed-In Buffalo Jump	49.749	-113.633
MZEUR-0353	Eurytoma spongiosa1	F	Ex. D. tumida on R. woodsii	J. D. & M.R. Shorthouse	10-May-2011	Alberta	Township Road 92, Willow Creek No. 26	Head-Smashed-In Buffalo Jump	49.749	-113.633
MZEUR-0354	Eurytoma spongiosa1	F	Ex. D. tumida on R. woodsii	J. D. & M.R. Shorthouse	10-May-2011	Alberta	Township Road 92, Willow Creek No. 26	Head-Smashed-In Buffalo Jump	49.749	-113.633
MZEUR-0355	Eurytoma spongiosa1	F	Ex. D. tumida on R. woodsii	J. D. & M.R. Shorthouse	10-May-2011	Alberta	Township Road 92, Willow Creek No. 26	Head-Smashed-In Buffalo Jump	49.749	-113.633
MZEUR-0356	Eurytoma spongiosa1	M	Ex. D. tumida on R. woodsii	J. D. & M.R. Shorthouse	10-May-2011	Alberta	Township Road 92, Willow Creek No. 26	Head-Smashed-In Buffalo Jump	49.749	-113.633
MZEUR-0357	Eurytoma discordans	M	Ex. D. tumida on R. woodsii	J. D. & M.R. Shorthouse	10-May-2011	Alberta	Township Road 92, Willow Creek No. 26	Head-Smashed-In Buffalo Jump	49.749	-113.633
MZEUR-0358	Eurytoma spongiosa1	M	Ex. D. tumida on R. woodsii	J. D. & M.R. Shorthouse	10-May-2011	Alberta	Township Road 92, Willow Creek No. 26	Head-Smashed-In Buffalo Jump	49.749	-113.633
MZEUR-0359	Eurytoma discordans	F	Ex. D. bicolor on R. woodsii	J. D. & M.R. Shorthouse	11-May-2011	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018
MZEUR-0360	Eurytoma iniquus	F	Ex. D. bicolor on R. woodsii	J. D. & M.R. Shorthouse	11-May-2011	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018
MZEUR-0361	Eurytoma discordans	F	Ex. D. bicolor on R. woodsii	J. D. & M.R. Shorthouse	11-May-2011	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018
MZEUR-0362	Eurytoma discordans	F	Ex. D. bicolor on R. woodsii	J. D. & M.R. Shorthouse	11-May-2011	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018
MZEUR-0363	Eurytoma iniquus	F	Ex. D. bicolor on R. woodsii	J. D. & M.R. Shorthouse	11-May-2011	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018
MZEUR-0364	Eurytoma discordans	F	Ex. D. bicolor on R. woodsii	J. D. & M.R. Shorthouse	11-May-2011	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018
MZEUR-0365	Eurytoma discordans	M	Ex. D. bicolor on R. woodsii	J. D. & M.R. Shorthouse	11-May-2011	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018
MZEUR-0366	Eurytoma discordans	M	Ex. D. bicolor on R. woodsii	J. D. & M.R. Shorthouse	11-May-2011	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018
MZEUR-0367	Eurytoma discordans	M	Ex. D. bicolor on R. woodsii	J. D. & M.R. Shorthouse	11-May-2011	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018
MZEUR-0368	Eurytoma discordans	M	Ex. D. bicolor on R. woodsii	J. D. & M.R. Shorthouse	11-May-2011	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018
MZEUR-0369	Eurytoma discordans	M	Ex. D. bicolor on R. woodsii	J. D. & M.R. Shorthouse	11-May-2011	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018

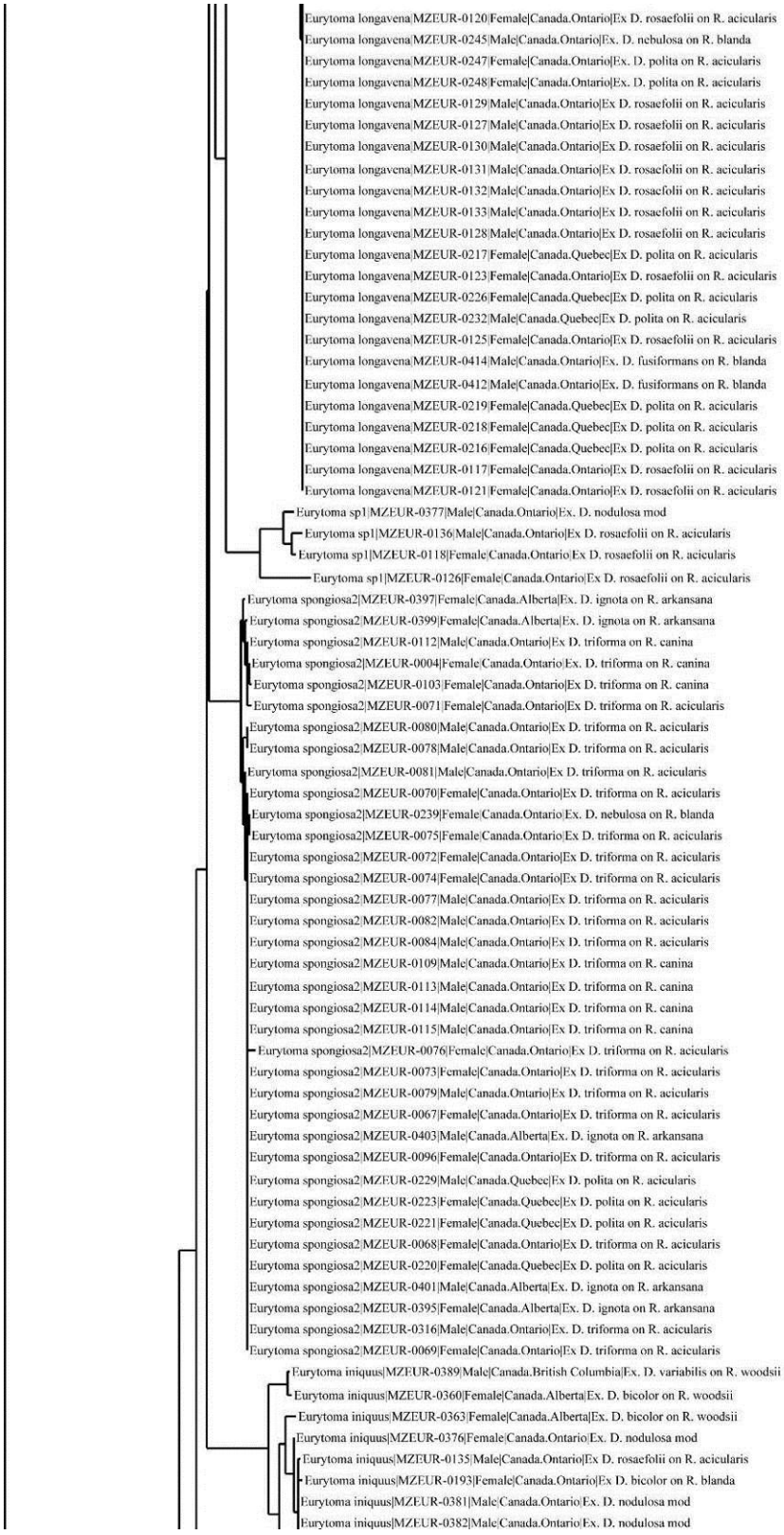
MZEUR-0370	Eurytoma discordans	M	Ex. D. bicolor on R. woodsii	J. D. & M.R. Shorthouse	11-May-2011	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018
MZEUR-0371	Eurytoma discordans	F	Ex. D. nodulosa mod	J. D. Shorthouse, B. L. Smallwood & M. Y. Zhang	29-Apr-2011	Ontario	Providence Bay	Beach	45.829	-82.337
MZEUR-0372	Eurytoma discordans	F	Ex. D. nodulosa mod	J. D. Shorthouse, B. L. Smallwood & M. Y. Zhang	29-Apr-2011	Ontario	Providence Bay	Beach	45.829	-82.337
MZEUR-0376	Eurytoma iniquus	F	Ex. D. nodulosa mod	J. D. Shorthouse, B. L. Smallwood & M. Y. Zhang	29-Apr-2011	Ontario	Providence Bay	Beach	45.829	-82.337
MZEUR-0377	Eurytoma sp1	M	Ex. D. nodulosa mod	J. D. Shorthouse, B. L. Smallwood & M. Y. Zhang	29-Apr-2011	Ontario	Providence Bay	Beach	45.829	-82.337
MZEUR-0378	Eurytoma discordans	M	Ex. D. nodulosa mod	J. D. Shorthouse, B. L. Smallwood & M. Y. Zhang	29-Apr-2011	Ontario	Providence Bay	Beach	45.829	-82.337
MZEUR-0379	Eurytoma discordans	M	Ex. D. nodulosa mod	J. D. Shorthouse, B. L. Smallwood & M. Y. Zhang	29-Apr-2011	Ontario	Providence Bay	Beach	45.829	-82.337
MZEUR-0381	Eurytoma iniquus	M	Ex. D. nodulosa mod	J. D. Shorthouse, B. L. Smallwood & M. Y. Zhang	29-Apr-2011	Ontario	Providence Bay	Beach	45.829	-82.337
MZEUR-0382	Eurytoma iniquus	M	Ex. D. nodulosa mod	J. D. Shorthouse, B. L. Smallwood & M. Y. Zhang	29-Apr-2011	Ontario	Providence Bay	Beach	45.829	-82.337
MZEUR-0383	Eurytoma discordans	F	Ex. D. variabilis on R. woodsii	R. G. Lalonde	20-May-2008	British Columbia	Kelowna	2km S. E. of Kelowna airport	49.952	-119.381
MZEUR-0384	Eurytoma discordans	F	Ex. D. variabilis on R. woodsii	R. G. Lalonde	20-May-2008	British Columbia	Kelowna	2km S. E. of Kelowna airport	49.952	-119.381
MZEUR-0385	Eurytoma spongiosa1	F	Ex. D. variabilis on R. woodsii	R. G. Lalonde	20-May-2008	British Columbia	Kelowna	2km S. E. of Kelowna airport	49.952	-119.381
MZEUR-0386	Eurytoma spongiosa1	F	Ex. D. variabilis on R. woodsii	R. G. Lalonde	20-May-2008	British Columbia	Kelowna	2km S. E. of Kelowna airport	49.952	-119.381
MZEUR-0387	Eurytoma spongiosa1	F	Ex. D. variabilis on R. woodsii	R. G. Lalonde	20-May-2008	British Columbia	Kelowna	2km S. E. of Kelowna airport	49.952	-119.381
MZEUR-0388	Eurytoma spongiosa1	M	Ex. D. variabilis on R. woodsii	R. G. Lalonde	20-May-2008	British Columbia	Kelowna	2km S. E. of Kelowna airport	49.952	-119.381
MZEUR-0389	Eurytoma iniquus	M	Ex. D. variabilis on R. woodsii	R. G. Lalonde	20-May-2008	British Columbia	Kelowna	2km S. E. of Kelowna airport	49.952	-119.381
MZEUR-0390	Eurytoma discordans	M	Ex. D. variabilis on R. woodsii	R. G. Lalonde	20-May-2008	British Columbia	Kelowna	2km S. E. of Kelowna airport	49.952	-119.381
MZEUR-0391	Eurytoma discordans	F	Ex. D. nodulosa mod	J. D. & M.R. Shorthouse	09-May-2007	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018
MZEUR-0392	Eurytoma discordans	F	Ex. D. nodulosa mod	J. D. & M.R. Shorthouse	09-May-2007	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018
MZEUR-0393	Eurytoma discordans	M	Ex. D. nodulosa mod	J. D. & M.R. Shorthouse	09-May-2007	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018

MZEUR-0394	Eurytoma discordans	M	Ex. D. nodulosa mod	J. D. & M.R. Shorthouse	09-May-2007	Alberta	Waterton Lakes National Park	Red Rock Canyon Road	49.093	-114.018
MZEUR-0395	Eurytoma spongiosa2	F	Ex. D. ignota on R. arkansana	J. D. & M.R. Shorthouse	12-May-2007	Alberta	Coaldale	Oldman River Valley	49.726	-112.619
MZEUR-0396	Eurytoma spongiosa1	F	Ex. D. ignota on R. arkansana	J. D. & M.R. Shorthouse	12-May-2007	Alberta	Coaldale	Oldman River Valley	49.726	-112.619
MZEUR-0397	Eurytoma spongiosa2	F	Ex. D. ignota on R. arkansana	J. D. & M.R. Shorthouse	12-May-2007	Alberta	Coaldale	Oldman River Valley	49.726	-112.619
MZEUR-0398	Eurytoma spongiosa1	F	Ex. D. ignota on R. arkansana	J. D. & M.R. Shorthouse	12-May-2007	Alberta	Coaldale	Oldman River Valley	49.726	-112.619
MZEUR-0399	Eurytoma spongiosa2	F	Ex. D. ignota on R. arkansana	J. D. & M.R. Shorthouse	12-May-2007	Alberta	Coaldale	Oldman River Valley	49.726	-112.619
MZEUR-0400	Eurytoma spongiosa2	F	Ex. D. ignota on R. arkansana	J. D. & M.R. Shorthouse	12-May-2007	Alberta	Coaldale	Oldman River Valley	49.726	-112.619
MZEUR-0401	Eurytoma spongiosa2	M	Ex. D. ignota on R. arkansana	J. D. & M.R. Shorthouse	12-May-2007	Alberta	Coaldale	Oldman River Valley	49.726	-112.619
MZEUR-0403	Eurytoma spongiosa2	M	Ex. D. ignota on R. arkansana	J. D. & M.R. Shorthouse	12-May-2007	Alberta	Coaldale	Oldman River Valley	49.726	-112.619
MZEUR-0404	Eurytoma sp3	M	Ex. D. ignota on R. arkansana	J. D. & M.R. Shorthouse	12-May-2007	Alberta	Coaldale	Oldman River Valley	49.726	-112.619
MZEUR-0412	Eurytoma longavena	M	Ex. D. fusiformans on R. blanda	J. D. Shorthouse	15-Apr-2000	Ontario	Renfrew	Roadside	45.472	-76.683
MZEUR-0414	Eurytoma longavena	M	Ex. D. fusiformans on R. blanda	J. D. Shorthouse	15-Apr-2000	Ontario	Renfrew	Roadside	45.472	-76.683
MZEUR-0415	Eurytoma spongiosa2	M	Ex. D. fusiformans on R. blanda	J. D. Shorthouse	15-Apr-2000	Ontario	Renfrew	Roadside	45.472	-76.683
MZEUR-0418	Eurytoma longavena	F	Ex. D. nebulosa on R. woodsii	J. D. Shorthouse	24-Oct-2002	Alberta	Coaldale	Oldman River Valley	49.726	-112.619
MZEUR-0419	Eurytoma longavena	F	Ex. D. nebulosa on R. woodsii	J. D. Shorthouse	24-Oct-2002	Alberta	Coaldale	Oldman River Valley	49.726	-112.619
MZEUR-0422	Eurytoma longavena	F	Ex. D. nebulosa on R. woodsii	J. D. Shorthouse	24-Oct-2002	Alberta	Coaldale	Oldman River Valley	49.726	-112.619
MZPAR-0003	Orthopelma mediator	M	Ex D. triforma on R. canina	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337

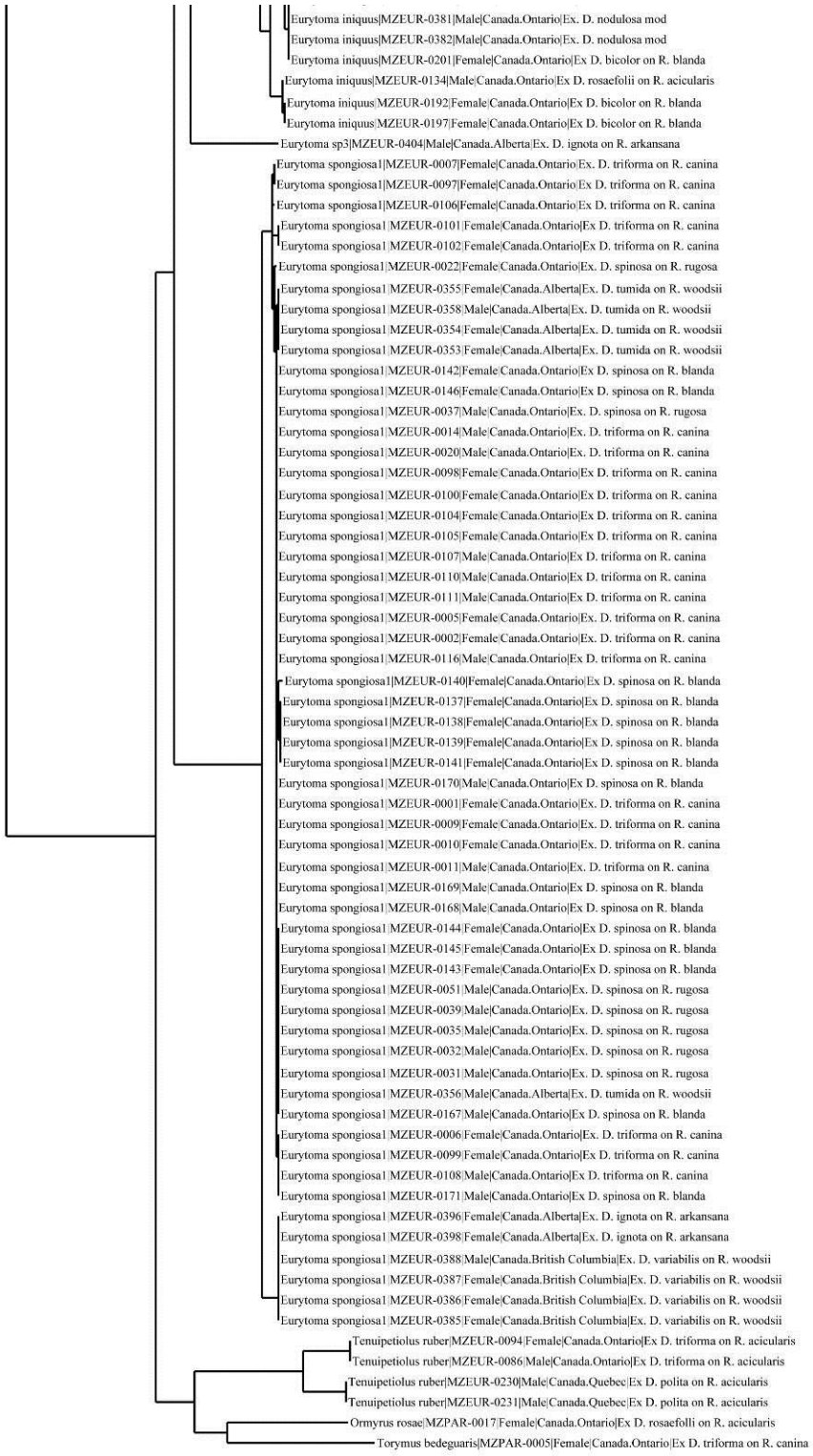
MZPAR-0005	Torymus bedeguaris	F	Ex D. triforma on R. canina	J. D. Shorthouse & J. D. Renelli	02-May-2009	Ontario	Manitoulin Island	Homestead near Lake Kagawong	45.829	-82.337
MZPAR-0017	Ormyrus rosae	F	Ex D. rosaefolli on R. acicularis	J.D. Shorthouse & M.Y. Zhang	24-Apr-2010	Ontario	Cochrane	Roadside near Genier	49.167	-81.017



Appendix II: Neighbour-joining tree for all available eurytomid COI sequences.

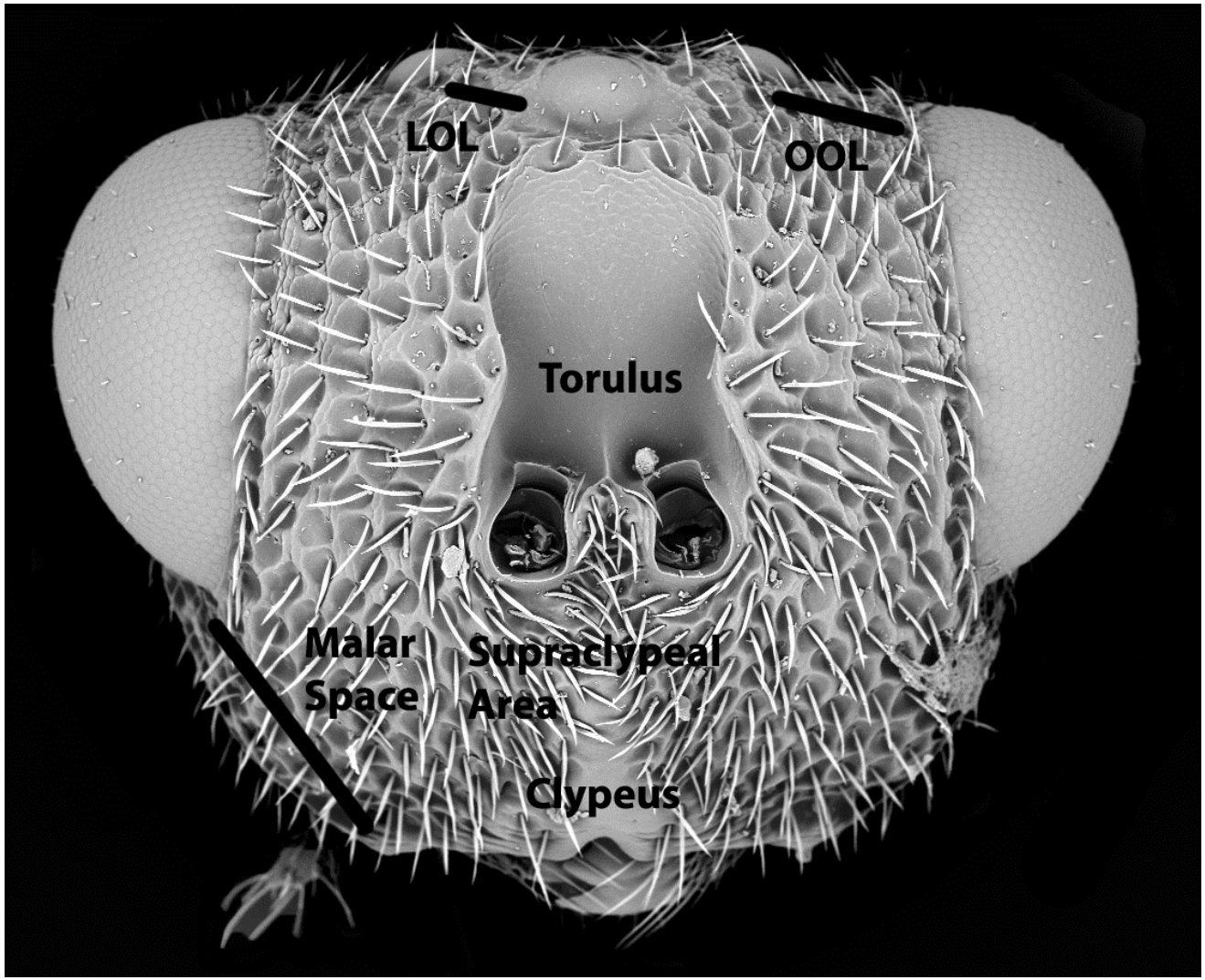


Appendix II cont'd: Neighbour-joining tree for all available eurytomid COI sequences.

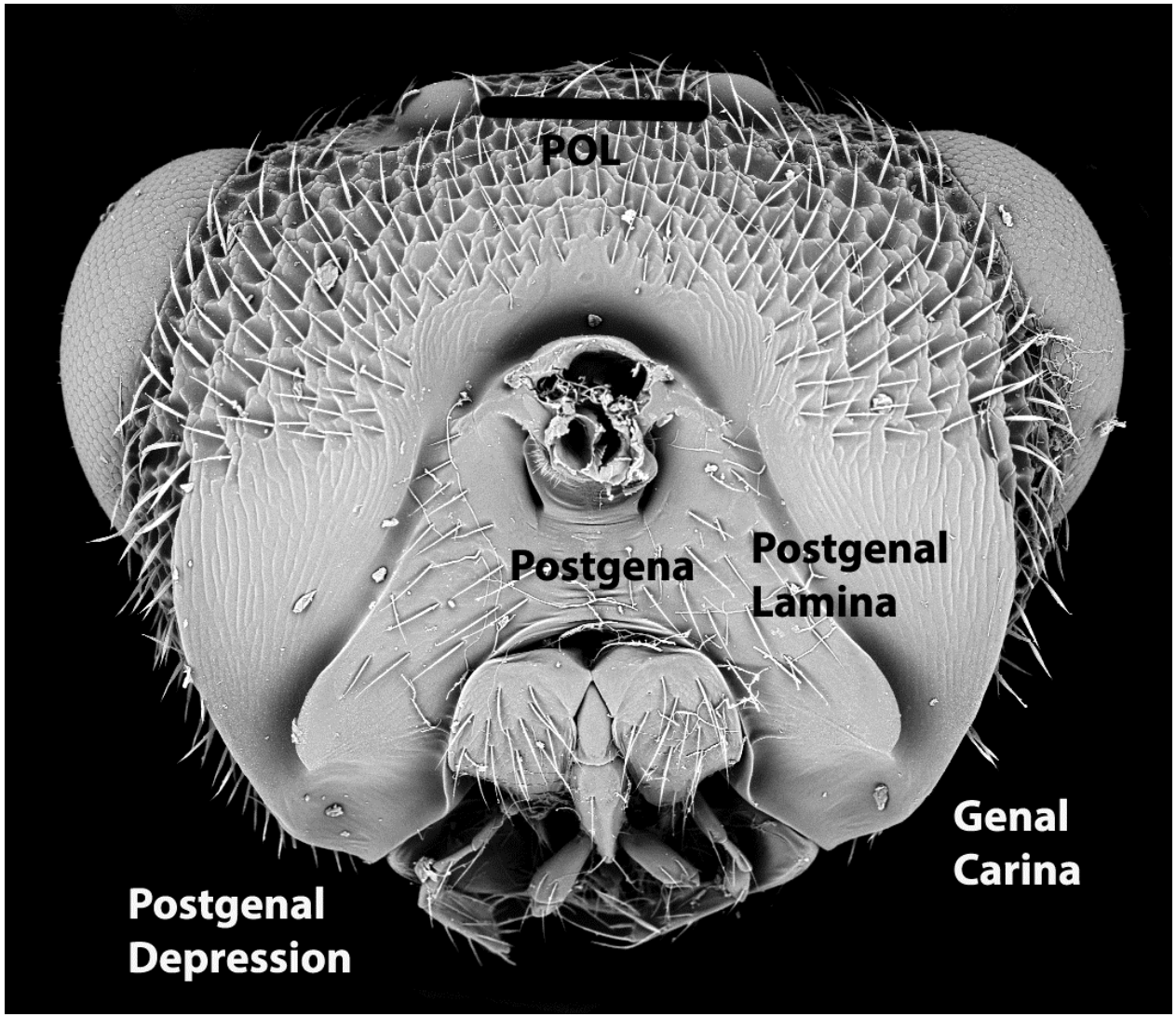


Appendix II cont'd: Neighbour-joining tree for all available eurytomid COI sequences.

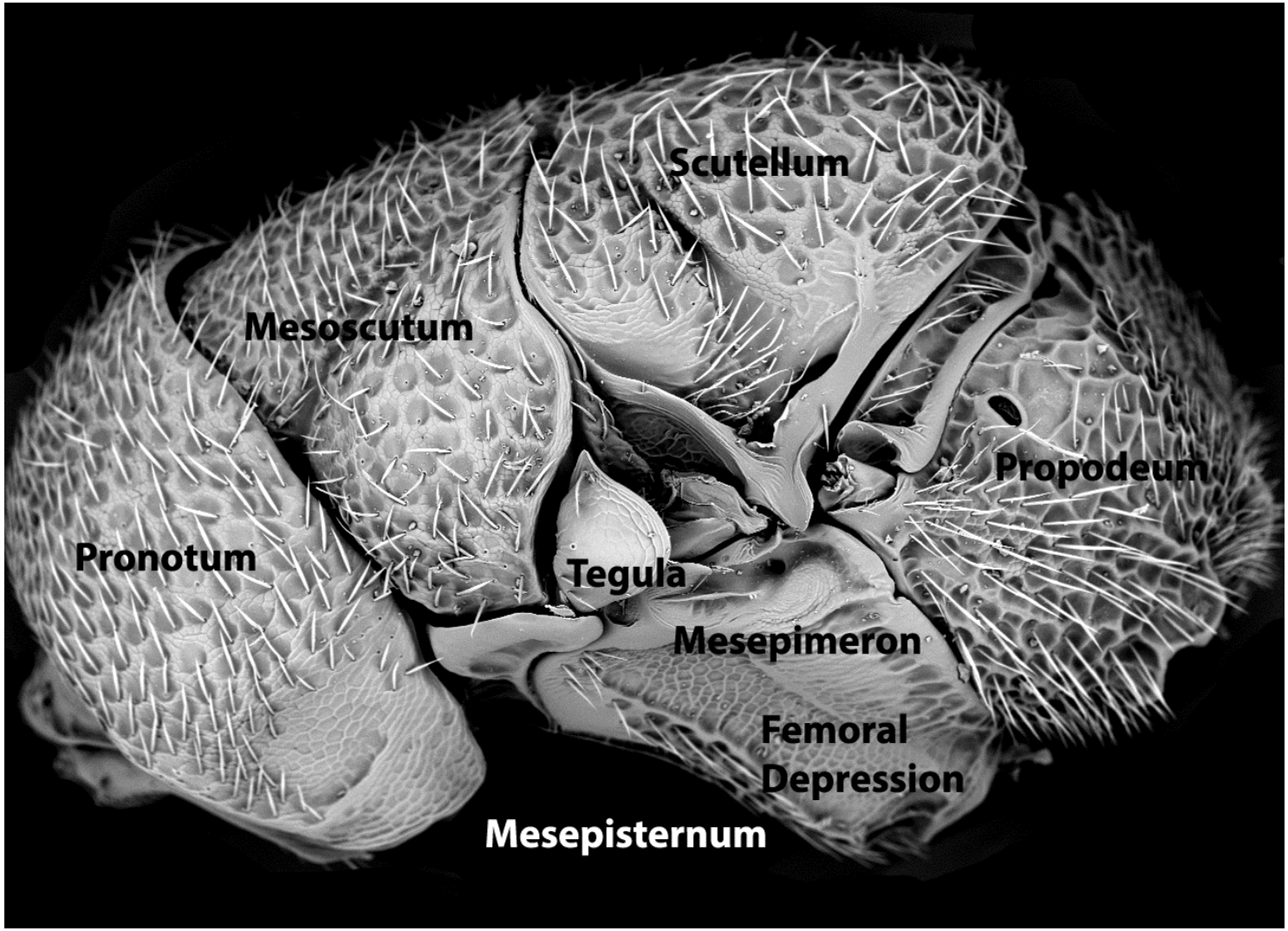
Appendix III: Anatomy of eurytomid



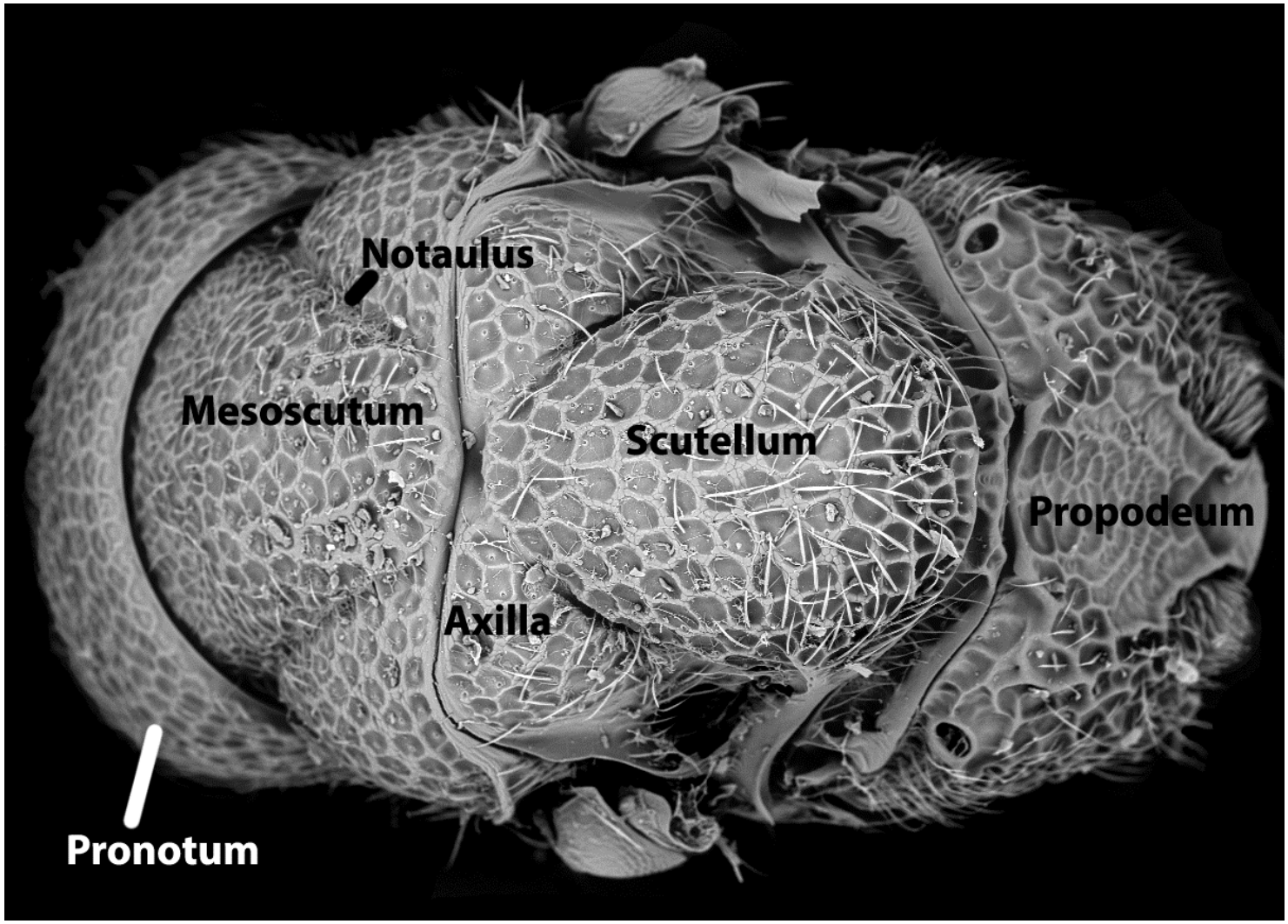
Appendix III-1. Head Anterior



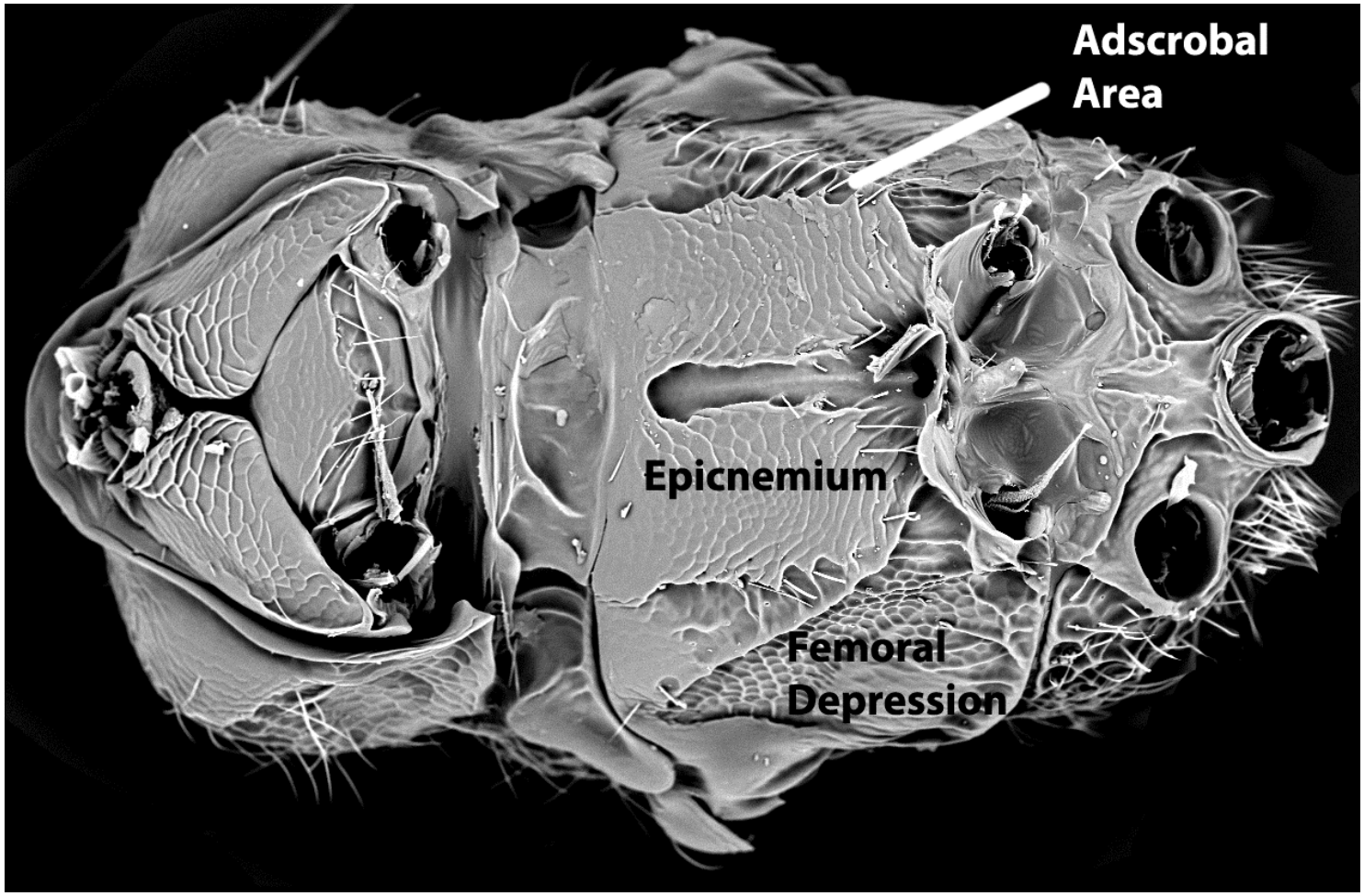
Appendix III-2. Head Posterior



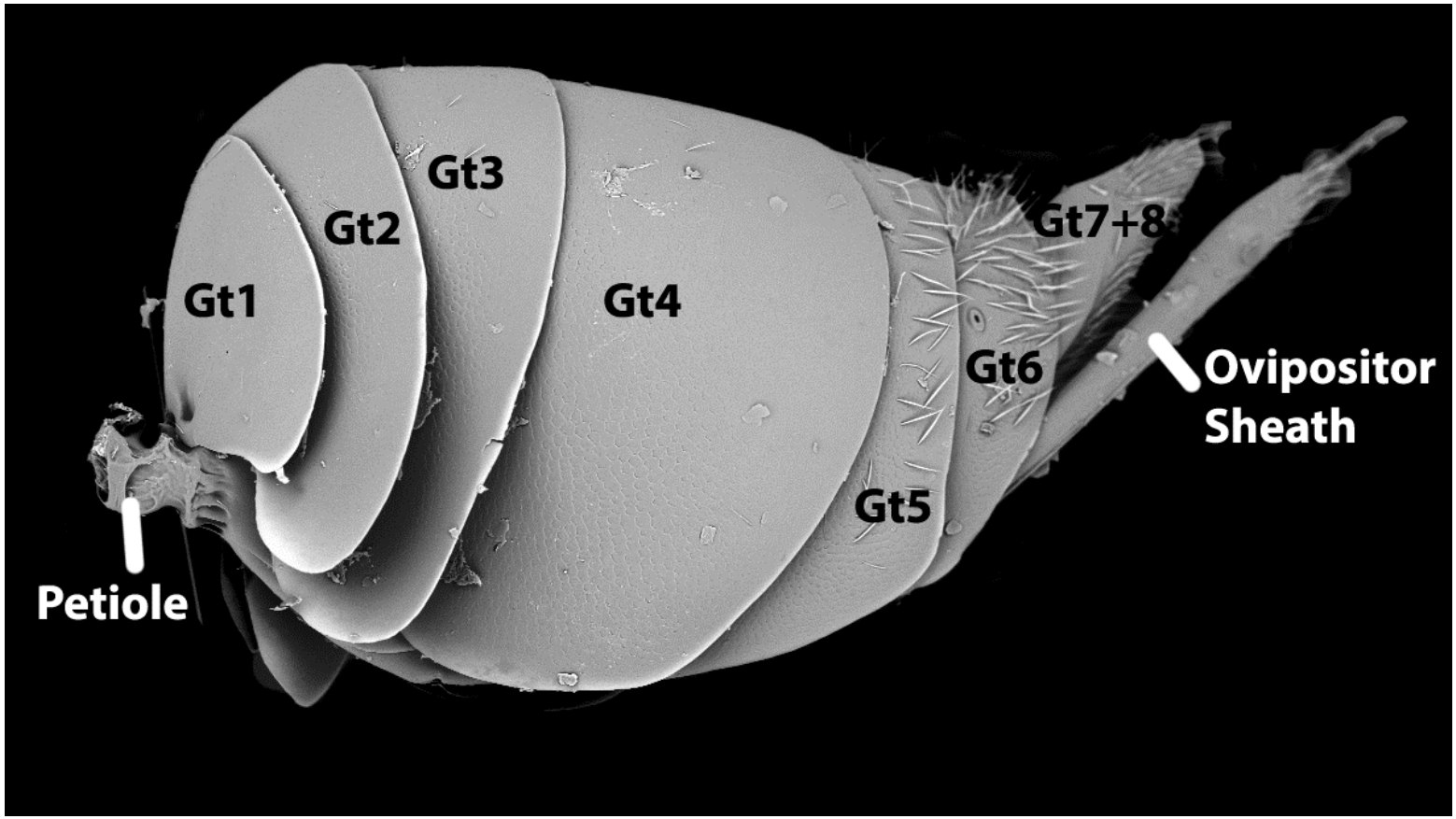
Appendix III-3. Mesosoma Lateral



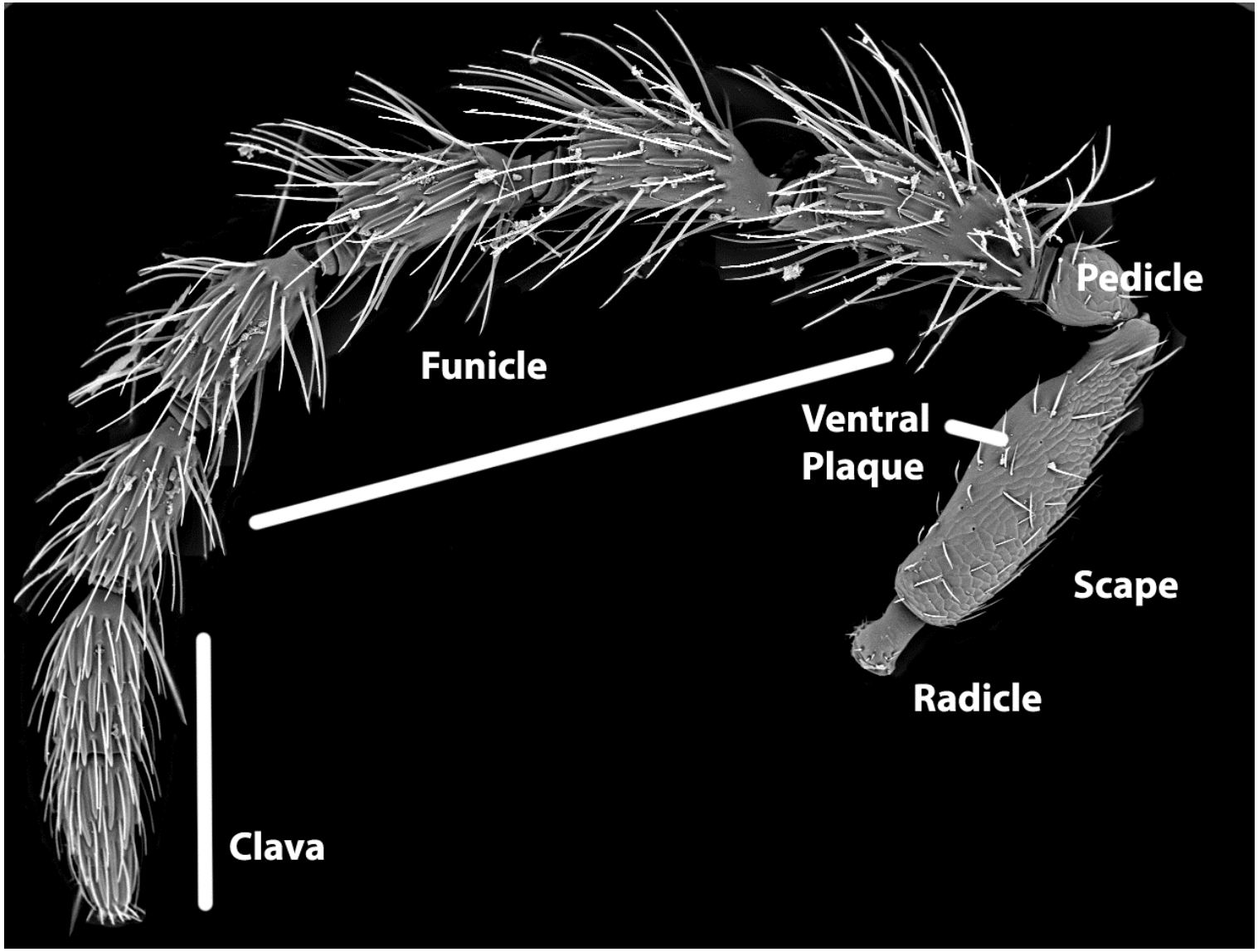
Appendix III-4. Mesosoma Dorsal



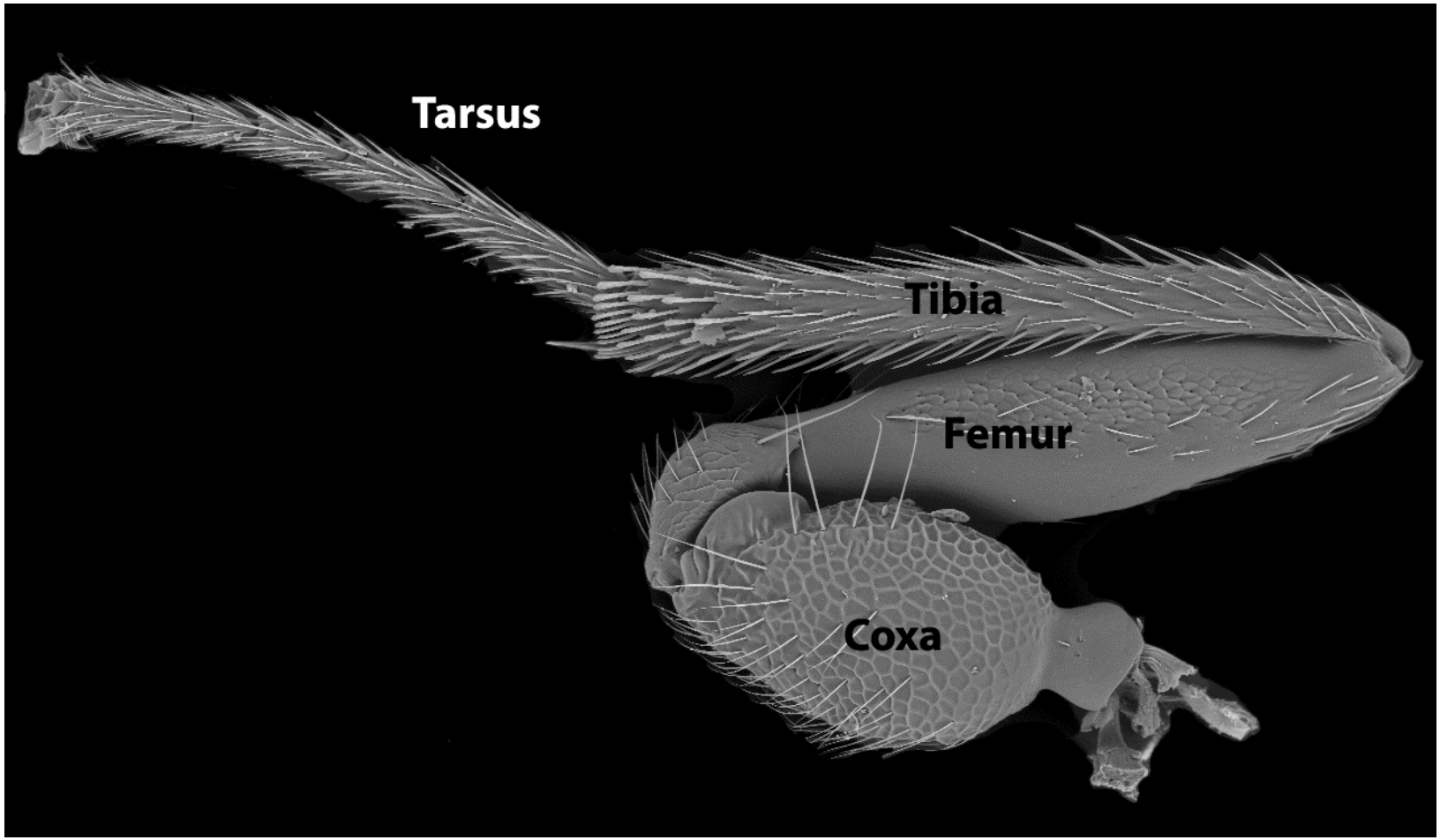
Appendix III-5. Mesosoma Ventral



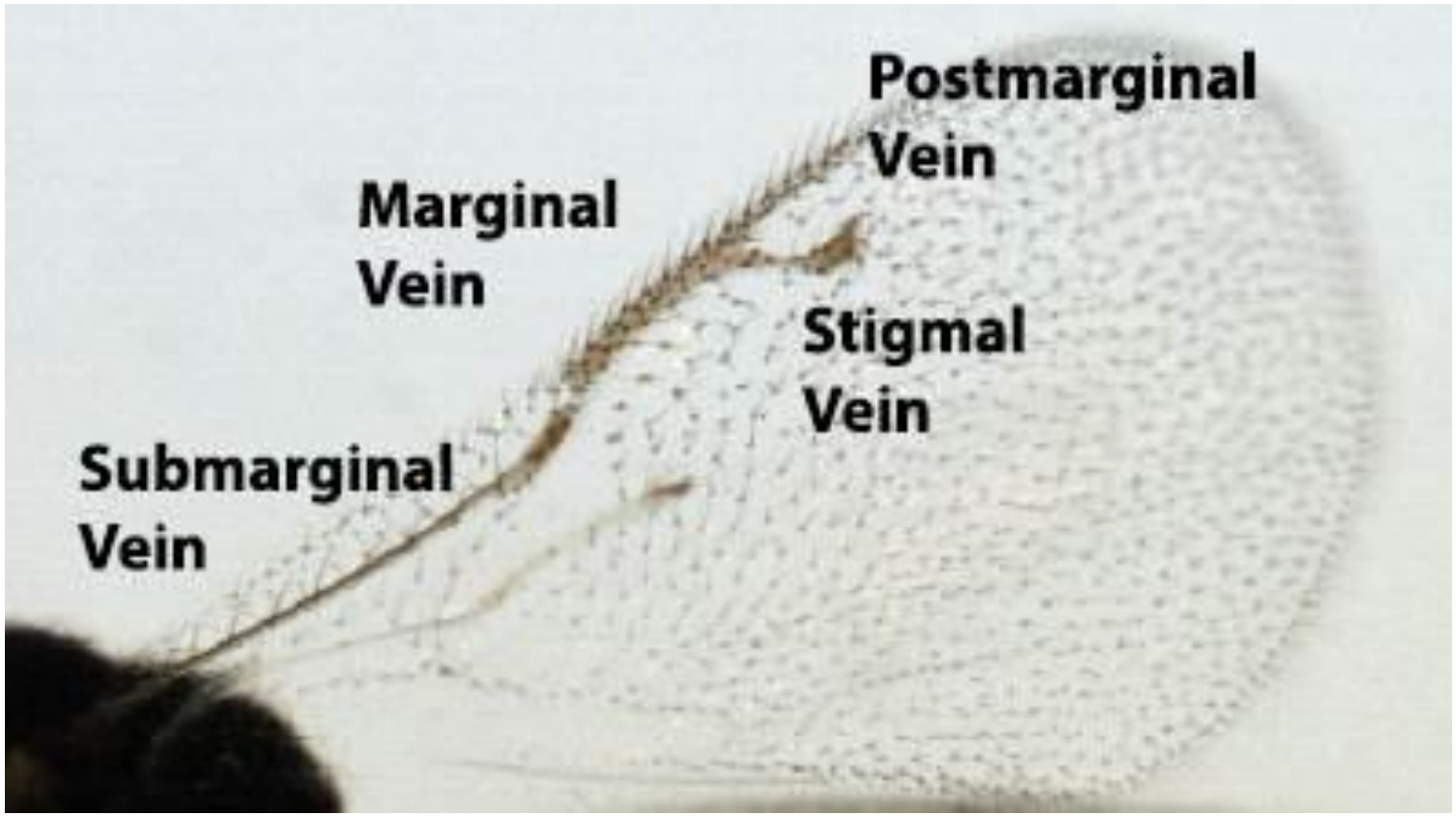
Appendix III-6. Female Metasoma Lateral


Pedicle
Funicle
Ventral
Plaque
Scape
Radicle
Clava

Appendix III-7. Male Antenna



Appendix III-8. Hindleg



Appendix III-9. Forewing